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SURVEY OF TECHNIQUES USED TO PRESERVE BIOLOGICAL MATERIALS

By

E. J. FEINLER and R. W. HUBBARD

January 1972

Prepared under Contract NAS2-6201 by

STANFORD RESEARCH INSTITUTE
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AMES RESEARCH CENTER
NATIONAL AERONAUTICS AND SPACE ADMINISTRATION

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I. INTRODUCTION AND METHOD OF APPROACH

Performance of research on living systems in space will require increasingly complex and varied methods of sampling and analysis. In-flight or real-time analysis is preferred, but priorities such as time, expense, adverse conditions, and availability of trained personnel will limit the amount of real-time analysis that can be done. Postflight or postponed handling of biological samples will be necessary. This introduces the problem of how best to store or preserve biological materials until they can be used.

The purpose of this report is to document and summarize existing techniques used to preserve biological materials. The report is presented in a handbook format that categorizes the most important preservation techniques available, and includes a representative sampling of the thousands of applications of these techniques to biological materials and organisms.

Details of the information coverage and method of approach are outlined in the following sections.

Literature Coverage

There is a vast amount of literature covering preservation of biological materials. Some preservation methods still in use today were originally described in the literature more than a century ago. Although there is a wealth of information available, most of it is poorly indexed or buried in books and articles whose main emphasis is not preservation *per se*. For these reasons, a variety of approaches were needed for searching the literature.

First, demand searches were requested from NASA, DDC, and MEDLARS. These were screened, and appropriate documents were ordered. Next, books, symposia, and reviews were covered through such sources as: *Books in Print*, *Cumulative Book Index*, *The Publishers' Tradelist Annual*, and the library catalogs of Stanford Research Institute, NASA Ames, and Stanford University. Few books were found that were directly related to the subject. Several books were found that had sections or chapters of interest, but these were not indexed under "preservation" headings and were therefore difficult to locate except by chance.

The bulk of the information presented here was found by searching the secondary abstracting sources. Again, the subject indexing was not adequate, and, in some cases, whole sections of the abstracting journal were scanned page by page to find useful information. Many author searches were also performed to obtain references to the work of recognized researchers.

The following abstracting journals were covered from approximately 1960 to June 1971 (unless otherwise noted):

Chemical Abstracts
Index Medicus
Biological Abstracts
Pandex (three years only)
Science Citation Index (three years only)
NLM Monthly Bibliography of Medical Reviews (two years only)

Pertinent abstracts were screened, and selected articles were ordered in hard copy or microfiche. When articles were received, they were separated into experimental or review papers and were further screened for inclusion in either the review section of the handbook, the data tables, and/or the bibliography. (Articles that were not included in either the review section or the tables due to various restrictions were still cited in the final bibliography, if of interest at all.)

Many of the bibliographies accompanying the articles were also checked for pertinent references, as were the tables of content of all issues of Cryobiology and several other relevant journals. In this manner, older information was obtained much more quickly than by searching the poorly indexed abstracting journals.

An overlap occurred between coverage in journal articles, symposium papers, and technical reports. Some authors published similar information in all three. The journal article was selected over the other two forms wherever possible, because journal literature was considered to be more easily obtainable by the user than report or symposium literature.

Subject Areas Not Covered

Because the literature covering preservation of biological materials is so prolific, it was impossible to cover all aspects of the subject within the scope of this report without making several restrictions. Therefore, the following information is not covered in this report:

(1) Information written in a foreign language was not included. However, many foreign authors are represented through translations and publications in English journals.

(2) Food preservation was not covered. This area is covered very well by the various abstracting services, books, and review sources such as Advances in Food Research. Many preservation techniques have been highly perfected for use on foods; however, the volume of literature on this topic was prohibitive for inclusion in a compilation of this scope.

(3) Histology and histochemistry were not included in the data compilation, again because the information is well documented elsewhere and the amount of material was prohibitive. Although

we did not include this information in the data compilation, many references on histology or histochemistry are included in the bibliography.

(4) Organic or biochemical substances are often lyophilized, dried, or crystallized during extraction or synthesis. Although these are forms of storage, this information was not covered in this survey unless the preservation method was the main emphasis of the paper in question.

(5) Most preservation by radiation was not included as the bulk of this information pertained to food or to simple sterilization.

(6) Preservation by packaging was not included.

Organization of the Handbook

General Organization

The handbook is organized into four main sections following this section:

(1) A review of reviews

(2) Tables of techniques of preservation

(3) Indexes

(4) A comprehensive bibliography

This format was chosen in an attempt to cover a variety of interests. The review section emphasizes evaluations and comparisons of work done in the preservation field and attempts to summarize findings, while the tables present a listing of preservation techniques and their applications, essentially as reported by the author with no attempt made to rate the relative merit of the technique or application. This approach should permit the user to evaluate the appropriateness of a technique or application for his own use, and also to see how it has been evaluated by other researchers in the field.

The bibliography contains all the references referred to in the review section and the tables, as well as many more related references. It is arranged alphabetically by author and chronologically under each author. This arrangement serves as an author index and also as a progress report on the work of a particular author.

In attempting to organize the information that had been gathered, it became evident that there were relatively few types of preservation methods in use, but that many variations and applications of each method existed. Therefore, it seemed logical to first categorize the material

by type of preservation method, and then by various applications of the preservation method.

The types of preservation technique were somewhat arbitrarily divided into the following categories:

- Freezing (temperatures below 0^oC)
- Refrigeration (temperatures around 4^oC)
- Lyophilization
- Chemical preservation (includes chemical sterilants, perfusates, various metabolic inhibitors, antioxidants, etc.)
- Drying and heat sterilization
- Ashing
- Fixation and embedding
- Radiation
- Dialysis
- Incubation

Details of the organization of each of the above-mentioned four sections that follow are outlined below.

Review of Reviews

The review of reviews section summarizes key information covered in over 100 review articles. It is organized by the technique categories outlined above. In addition, the last section of the review discusses applications of these techniques to mammalian specimens.

An attempt was made to provide sufficient information about each review to give the user an indication of what material is covered in the original article. Each reference mentioned in the review section is included in the bibliography and is preceded by a double asterisk.

Tables of Preservation Methods

The handbook tables are arranged first by the type of preservation technique, next by the kind of material being preserved, and, finally, chronologically under each kind or material.

The preservation technique is listed in the upper left-hand corner of each page of the tables in bold-face type. Directly under the preservation technique arranged horizontally across the page in columns are

listed the various pieces of information that were extracted from each paper. These columns are:

(1) Specimen Type. This gives a gross description of the type of material being preserved. The specimen types are listed alphabetically, and this list can be used as an index to the applications of each preservation technique under which the listing occurs.

(2) Original Form of Sample. This column describes the form of the sample at the time it was preserved, and also describes the genus-species name of the organism or the origin of the sample.

(3) Brief Description of the Method. This column provides a brief abstract of the preservation technique and details of its specific application.

(4) Limitations of the Method. This column outlines the effects that the preservation method has on the specimen being preserved. It covers such concepts as viability, unusual effects, limitations, restrictions, length of preservation time, etc.

(5) Other Comments About Method. This column presents additional information of interest, such as unusual observations, authors' comments, explanatory notes, etc.

(6) Estimated Preservation Time. The figures listed in this column give a rough estimate of how much time is required to preserve the specimen in question.

(7) Survival Time. These figures give an approximate length of time that the specimen can be stored, if preserved by the technique outlined. These figures are inexact, and they do not necessarily indicate that the specimen can be stored without damage for the length of time indicated. However, these figures should give an estimate of how long a sample preserved by a given technique can be kept.

(8) Estimated No. of Steps. A "step" is defined as one operation, such as injecting an animal, rather than one movement. These figures are relative and are included to give the user an indication of the complexity of the method.

(9) Equipment and Reagents Used. This column includes general equipment and reagents used during the preservation process.

(10) End Use of Specimen. This column gives the intended use or the method of analysis for the preserved specimen.

(11) Reference. Only the first author's last name and the date are included here. The full reference can be found in the bibliography and is preceded by a single asterisk.

An attempt was made to present enough information in the tables to allow the user to decide whether the method is useful for his purposes, and to make at least gross comparisons among the different methods and applications.

The left-hand column of specimen types is arranged alphabetically under each preservation technique. This alphabetic listing can be used as a rough index. Where more than one specimen type of the same kind exists, the entries are arranged chronologically. This arrangement lets the user follow chronological developments in preserving a particular kind of material by a particular technique.

Most papers are entered only once in the tables. If more than one preservation technique was covered in a single paper, the information was entered in the tables under what seemed to be the most important technique. However, all preservation techniques are entered in the index. By using either the tables or the index, the user should be able to pinpoint most any information covered.

Many words were abbreviated in the tables to save space. A list of the abbreviations used is given in the front of that section.

Indexes

The indexes include the preservation technique(s), the material being preserved, biochemical or other quantities affected by the preservation technique, and reagents important to the technique.

A genus-species name is included in the index, if one was given in the original paper. If not, the animal or plant is indexed by its common name combined with the type of specimen being preserved. (For example, an entry involving preservation of rat kidneys would be entered under "Kidney" and under "Rat kidney" rather than under "Rat" alone.

Chemical nomenclature is presented for the most part as it appears in the original paper. Organic chemical entries are not inverted.

Bibliography

The bibliography is a composite of the references cited in the review section and the tables, but it also contains a great many references of interest that were not included in either of these sections. References cited in the review section are marked with a double asterisk (**), references cited in the tables are marked with a single asterisk (*), and references not included in either of these sections are unmarked.

The bibliography is arranged alphabetically by the first author's last name, then chronologically under each first author. This arrangement not only provides an index to authors in the field, but it also gives a brief summary of the sequence of work done by a given author.

The use of all these sections together should give the user a variety of approaches to methods of preservation of biological materials.

This compilation does not claim to be a complete compendium of preservation methods. Such a work could easily fill several volumes. Rather it is hoped that the most commonly used preservation methods are represented here, along with several applications for each method.

II. REVIEW OF REVIEWS

Introduction

Since review articles were more apt to summarize methodology rather than present experimental detail, they did not fit into the tables of data as readily as experimental papers. For this reason, review articles have been summarized separately here.

This section of the handbook outlines the key points of more than 100 reviews, including books. It is organized primarily by preservation technique except for the last section which summarizes applications of the various techniques to mammalian systems.

Freezing of Biological Samples

The term "freezing" is defined here as hardening into a solid body by extraction of heat. This phenomenon occurs in the vicinity of $0^{o}C$ for aqueous solutions. However, freezing temperatures as low as $-200^{o}C$ are often used for preservation of biological materials.

Freezing can slow, stop, or accelerate biochemical reactions. It can also preserve or disrupt the fine structure of biological cells. Which of these responses occurs during a given type of reaction or within a given cell depends on a variety of factors (Mazur, 1970).

If the freezing process is relatively slow within a biological system, ice crystals seem to form exclusively in the extracellular space (Mazur, 1970). Water within the cell becomes supercooled and, in this state, has a higher vapor pressure than ice. This intracellular water is then withdrawn from the cell to contribute to ice crystal formation, which finally consumes all freezable water in and out of the cell except for 5 to 10% of the intracellular water remaining in the cell unfrozen.

Rapid cooling produces more and smaller ice crystals, which are predominantly intracellular. In general, very slow freezing and very rapid freezing are lethal to most types of animal cells (Mazur, 1970). It is possible that injuries produced in living cells by extracellular freezing may result from critical dehydration of the living protoplasmic system (Asahina, 1965). The resulting deleterious effect of concentrated salt solutions on the protoplasmic membrane is well known (A. Smith,1961).

In 1940, it occurred to Luyet and Genehio that crystallization of ice could be prevented by ultra-rapid cooling of minute amounts of fluid to a very low temperature, so that the water molecules would have no time to form crystals of ice, or the liquid become vitrified. If the

glassy material was subsequently rewarmed at an ultra-rapid rate, it should revert to the liquid state without ice formation. This approach only worked on a very limited micro-scale (G. Smith, 1950). In general, as cells are cooled below $0^{o}C$, they become subject to three phenomena: the temperature falls; ice crystals form; and liquid water is removed thus raising the concentration of solutes. Most of the freezing injury to biological cells appears to be due to the combined effects of exposure to concentrated solutes and the formation of large intracellular crystals (Richards, 1964; Mazur, 1965).

Epithelium, some tumor tissues, and erythrocytes are cellular types that will tolerate very rapid freezing. Optimum cooling velocities to give the best cell survival vary from $1.6^{o}C$ per minute for bone marrow stem cells to about $3000^{o}C$ per minute for human erythrocytes (Meryman, 1964; Mazur, 1966; Luyet, 1970). The optimum freezing rate for each cellular type minimizes both the intracellular ice formation and the solution effects (Luyet, 1970). Preservation of whole human skin for grafting purposes for periods of 1 to 61 days has been best accomplished with slow freezing and rapid thawing (Perry, 1966, 1966a; Ballantyne, Jr., 1966). The epithelial or outer layer of skin and whole skin, therefore, appear to respond differently to freezing and thawing for cellular survival. This is not surprising because of the greater number of different types of cells (each with different freezing and thawing requirements) found in whole skin compared with the epithelial layer alone.

The use of protective additives--such as a 5 to 20% concentration of glycerin, dimethylsulfoxide (DMSO), or polyvinylpyrrolidone (PVP)--prevents a significant amount of water from freezing and thus gives a much greater chance for cellular survival (Huggins, 1965). Practical methods for the removal of these additive agents after thawing, to allow for either utilization of the cellular materials in biological systems or further analysis of these materials, have been developed (Huggins, 1969). The need to remove glycerol, especially from blood cells, arises from the fact that the rate of water exchange across the red cell membrane is greater than the glycerol exchange, thus giving rise to an osmotic imbalance that could lyse the cell (Pyle, 1964). PVP produces a better survival rate for marrow cells and red cells than does glycerol, and PVP does not usually have to be removed before returning the cells to a physiological environment (Richards, 1964). All these cryoprotectants appear to exert protective action by reducing the concentration of salt in equilibrium with ice at any given temperature, thus decreasing the likelihood of denaturation of proteins and other complex organic substances in the external and internal membranes and organelles of living cells (G. Smith, 1950). These cryoprotectants appear to interact directly with the hydration shell of biologically important macromolecules and thereby influence macromolecular conformation (Doebbler, 1966; Rowe, 1966; Karow, Jr., 1969a).

Studies concerning the long term preservation of whole blood and red cells by freezing have been extensive (Tullis, 1966; Valeri, 1966, 1968, 1968a; Meryman, 1968). Recent advances in preservation of blood

by freezing and its clinical acceptability have been outlined (Valeri, 1968, 1968a). Erythrocytes recovered physically intact after rapid freezing have their electrolyte composition altered. Total cation concentration remains unchanged, while sodium increases and potassium decreases (Doebbler, 1965). This electrolyte change may be due to an increased activation after freezing of nucleoside triphosphatase activity, which is involved in erythrocyte membrane cation transport (Doebbler, 1965).

Other blood cells such as platelets have shown a very poor recovery rate after freezing (Gardner, 1968). Even the initial centrifugation to prepare platelet-rich plasma causes a 25% loss of platelets. The use of glycerol for platelet protection during freezing alters the platelet membrane before freezing and decreases the recovery of circulating platelets that can be recovered (Gardner, 1968). The use of radioactive sodium chromate (Cr^{51}) has proved to be a successful platelet marker for measuring platelet survival (Aas, 1958).

It is suggested that a separation of marrow cells into component cells be attempted before freezing, so that the components may be studied separately (Ashwood-Smith, 1965). Although frozen marrow may have less antigenicity than fresh material, it is unlikely that a freezing method will be devised to enable marrow to be as good after freezing as before (Ashwood-Smith, 1964). Leukocytes or the white cells of whole blood have been frozen in 10% DMSO, and the surviving cells were evaluated (Cavins, 1968). Unfortunately, DMSO causes a human toxic response, which means that it must be almost totally removed from the preserved material if it is to be placed back in the human body (Gardner, 1968). Efforts to preserve human leukemic white blood cells <u>in vitro</u> for experimental purposes have been described (Shohet, 1967). Salient features of this technique include slow freezing, rapid thawing, the use of DMSO as cryoprotective agent, and brief exposure of the thawed cells to deoxyribonuclease. Overall recovery of viable cells was over 50%.

Antigens and antibodies that are constituents of blood serum can be preserved at -79°C for many months with no alteration in antigenic properties (Greaves, 1965; Krijnen, 1968). Greaves (1965a) states that freeze-drying is a more complex technique than simple freezing, and that it is also potentially more destructive. He also points out that the exact point of complete freezing can be detected using the electrical resistance of ice in an A-C conductivity cell.

Preservation of serum plasma by freezing illustrates the well-known lipid insolubility effect of freezing and thawing (Pennell, 1965; Greaves, 1968; Davies, 1968; Martinek, 1970). Lipoproteins have long been known to be most sensitive to freezing and thawing (Pennell, 1965), but urea and alkaline phosphatase activity levels are also adversely affected (Davies, 1968). In this case, lipoproteins are put into an insoluble state that causes the plasma to become cloudy after thawing. This cloudiness can be removed by suitable filtration (Greaves, 1968).

Successful freezing of large and complex organs is unlikely, owing to the number of different cells, each probably requiring a different freezing schedule (Greaves, 1965a). Certainly no mammalian heart has survived the rigors of freezing and thawing (Childs, 1969), but some fairly simple organ systems--such as cornea (Childs, 1969), when frozen to -195°C in the presence of 14% DMSO, serum, and sucrose--appear to be quite well preserved. With the corneas, freezing was done by degrees first to -80°C, then to 160°C, and then to -196°C (Pakarinen, 1969). Preservation of kidney, small intestine, lung, heart, and liver have all been attempted with varying degrees of success (Pakarinen, 1969). Refrigeration techniques and oxygen control have been tried more frequently than freezing (Norman, 1968; Pakarinen, 1969; Malinin, 1970). The many problems of organ storage by freezing--particularly of kidney, cornea, smooth muscle, and cartilage--are reviewed by A. Smith (1965), Martin (1968), and Halasz (1970). Freezing has not yet proven to be an effective way to preserve organs or, for that matter, adult mammalian cells, with the exceptions of a few cell types such as erythrocytes and sperm (Dolan, 1965; Abbott, 1969).

Assessment of tissue, organ, or cell viability has been well defined with divisions of apparent death, relative death, and absolute death (Malinin, 1967). The basic vital characteristic is the ability to take free energy from the environment and make it available for various synthetic processes (Dolan, 1965a).

Bull semen frozen to -196°C and kept for periods of at least ten years can still be used for artificial insemination (Sherman, 1965). However, aging of sperm is known to cause an increase in the incidence of embryonic or early fetal death (Sherman, 1965). Extensive studies of frozen human sperm have been made (Sherman, 1963), and viability of the frozen cell is also of primary interest (Wolstenholme, 1970).

Cryopreservation of bone or osseous tissue at -1° to 7°C in various media--such as saline, citrated blood, Ringers solution, and merthiolate--has been done for at least 40 years (Boyne, 1968). Deep frozen bone homografts appear to be superior clinically and histologically to those maintained at 4°C in any of the above-mentioned media. Freeze-drying has also been used successfully for this application. Sterilization by irradiation has been practiced extensively on bone homografts, as has chemical sterilization with ethylene oxide and beta-propiolactone (Boyne, 1968).

Going down the phylogenetic scale from vertebrates through invertebrates to microorganisms and viruses, preservation by freezing becomes less difficult. However, like the higher animal cells, most protozoa and some bacteria require cryoprotectants such as DMSO to prevent extensive freezing injury (Meryman, 1963). Frozen protozoa have been stored successfully at temperatures as high as -19°C and as low as -196°C (Diamond, 1964). In general, the lower the storage temperature employed, the longer the period of survival. Thawing is usually done as rapidly as possible (Diamond, 1964). Microorganisms are, in general,

more resistant to freezing than other highly developed animal or plant cells and are easier to preserve in the frozen state than other animal cells (Nei, 1964). Culture collections of microorganisms may be stored at low temperatures and used whenever desired (Boyne, 1968; Nei, 1965, 1969). Declines in virus titers associated with velocities of freezing of the order of 60°C per minute or greater were lower than those found with velocities of freezing of 40°C per minute or less (Greiff, 1965). Such freezing stability data for viruses have been established by group classification for refrigeration, freezing, and freeze drying methods of preservation (Rightsel, 1967).

The preservation of plant tissues by freezing has also received considerable study. It can be demonstrated that freezing alters the permeability properties of plant cell membranes (Heber, 1968). However, plant cells have the ability to synthesize specific protein factors that can protect their membrane system against freezing injury (Heber, 1968; Steponkus, 1969). This freezing protection coincides with an increase in ribonucleic acid and protein synthesis in autumn during the frost-hardening period of certain types of plants (Levitt, 1964, 1966). Frost or freezing injury in plant cells can be measured by the amount of amino acids and other ninhydrin reactants released from frozen cells (Reeve, 1966).

Many reviews concerning the general aspects of the biological effects of freezing on living cells appear in the literature (A. Smith, 1961; Luyet, 1965; A. Smith, 1970; Wolstenholme, 1962, 1970). Specific studies also appear on practical applications of low temperature preservation for cultured animal cells (Scherer, 1965).

A very special part of the living cell, the nucleus, has also been extensively studied (Stowell, 1965). The best structural preservation of nuclei has been attained with the most rapid possible cooling from 0°C to -100°C. Slow freezing leads to large, intranuclear, ice crystal formation during the change to the frozen state, with marked displacement of the nucleoplasm, including chromatin and nucleoli. Rapid thawing gives amazing structural reconstitution of the nucleus with only slight clumping of chromatin. Slow thawing, on the other hand, may give large residual areas of displaced nucleoplasm and changes in the nuclear envelope with general separation of the inner and outer nuclear membranes (Stowell, 1965).

Cellular damage by freezing appears to be caused by alterations of intra- and extracellular water. Bound water in the form of lattices seems to be essential to cell integrity, especially protein structure and function. Death by freezing seems to occur primarily as a result of extraction of bound water from vital cellular structures (Karow, Jr., 1965).

Electron microscopy is a valuable tool for the study of the fundamental mechanisms of biological freezing at the molecular level (Fernandez-Moran, 1960). The direct ultrastructural changes induced

in tissue by cold exposure must be directly visualized. Conventional thin section techniques cannot achieve this, but freeze substitution or "freeze-cleaving" or "freeze-etching" eliminates some of the artifact-producing steps of the thin section technique (Weinstein, 1967; Steere, 1969). Freeze substitution has been used on specimens of yeast cells, bacteria, algae, nematodes, mouse brain, parasitized red blood cells, and virus-infected tobacco cells (Steere, 1957, 1969).

The art of food preservation by freezing presents information that is of potential value to the preservation of all biological samples. The preservation of meat by freezing still offers the best organoleptic suitability over long periods of time (Lawrie, 1968). However, freezing of food does have some detrimental effects as far as the rate of freezing and thawing, as well as the use of chemical additives, are concerned; these are critical factors to consider in minimizing undesirable degradation (Fennema, 1966).

Lyophilization or Freeze-Drying

Lyophilization is the creation of a stable preparation of a biological substance by rapid freezing and dehydration of the frozen product under high vacuum.

Initial freezing of the sample must be done in a suitable low temperature medium, such as a mixture of dry-ice and acetone, followed by dehydration by sublimation from the frozen state. Heating the frozen sample to just below the melting point, as soon as the vacuum is attained, gives the most significant rate of sublimation. Sublimation starts at the ice surface and works progressively to the center or bottom. The amount of heat applied is significant because of the intense absorption of heat caused by sublimation. The amount of heat required during sublimation varies as the process continues, because the thermal gradient through the ice layer changes with the changing depth of ice (Chambers, 1949; Mullin, 1955; Flosdorf, 1959; Simatos, 1965).

Sublimation temperatures for most biological products such as blood serum, organic compounds, and bacterial suspensions are between -10° and $-40^{\circ}C$. This requirement can decrease to $-100^{\circ}C$ for cells where large ice crystal formation must be avoided (Chambers, 1949; Simatos, 1965).

Secondary drying after sublimation is usually done under high vacuum, or at slightly above room temperature, to lower residual water content of the sample to a minimum. However, the viability of certain bacteria and viruses is preserved only if their water content is kept above certain minimum levels (Sherman, 1963).

Most lyophilized samples are most effectively stored under nitrogen because of the very high porosity of the freeze-dried product (Simatos, 1965; Mullin, 1955).

Reconstitution of lyophilized biological samples commonly consists of rehydration with water or physiological saline. In many instances, the reconstituted product can be used in a more concentrated state than the initial product (Simatos, 1965).

The optimal freeze-drying temperature can be predicted for a given type of sample. Automatic control equipment can be used to measure this temperature by the use of electrical resistance measurements of the sample (Greaves, 1962, 1965).

The suspension medium used to freeze-dry a sample is very important for long term storage stability. Many different media have been reported to have been used successfully such as serum, broth, skimmed milk, and gelatin (Greaves, 1962).

The importance of mechanical design in lyophilization equipment cannot be minimized. Vapor paths must remain open, and differential pockets of high pressure must be avoided. Heating during lyophilization must not cause local melting. Constant removal of dried products offers a very fast drying rate. High vacuum spray freeze-drying has certain advantages in drying foods, if flavor and solubility are not critical, as both of these properties are sometimes altered by this technique (Mullin, 1955; Flosdorf, 1959; Greaves, 1962).

It is evident that the rate at which a solution freeze-dries depends on the temperature and the nature of the solute. Freeze-drying velocity is not always determined by the size of ice crystals which would in turn determine the size of vapor flow channels vacated by the subliming ice (Mullin, 1955; MacKenzie, 1965).

Mammalian spermatozoa and erythrocytes have both been freeze-dried with a significant percentage of survival (Meryman, 1963). A variety of mammalian serum protein fractions have also been freeze-dried (Rosenberg, 1964). Many different physical mechanisms have been employed to freeze-dry these serum proteins such as tray-drying, where the material has to be scraped from the trays and pulverized, and spray atomizing, where the frozen particles are transferred to a wire mesh drum mounted horizontally in a vacuum chamber with controlled radiant heat to insure operation just below the melting point (Greaves, 1960; Rosenberg, 1964). If a protein solution could be well preserved by freeze-drying without denaturation, this would probably be the method of choice, as it avoids the inconvenience of frozen storage (Rosenburg, 1964).

With more highly organized tissues and cells, the act of cooling itself can be expected to induce dislocations in interrelated enzyme systems due to the differing temperature coefficients of these systems (Rinfret, 1962). Alteration of aldolase activity has been reported in mammalian blood serum stored at low temperatures (Lehmann, 1965). Freeze-drying of nonviable human tissues, as developed by the Tissue Bank of the U.S. Naval Medical School, has permitted long term storage (several years) of these tissues in a state suitable for homotransplantation

(Gresham, 1964). The primary advantage of freeze-drying over the usual freezing methods for bone is the avoidance of prolonged low temperature storage (Pappas, 1968). Also, some differences in graft acceptance between freeze-dried and frozen preparations may eventually be demonstrated (Pappas, 1968).

Many viruses and bacteria can be very satisfactorily preserved by freeze-drying after initial freezing to -40°C and drying at -30°C. The use of 5% sodium glutamate and 10% purified bovine albumin in distilled water or isotonic saline has increased the yield of viable microorganisms that can be preserved by the freeze-drying process (Meryman, 1963). Most viruses will withstand freeze-drying (Burns, 1964), but the unfreezable water of cells plays an important role in cell viability during the freeze-drying of microorganisms (Nei, 1964). Lyophilization is often stated to be the most important method for the preservation of viruses. Viruses that are lyophilized are usually stored at 4°C but, in many other instances, may be stored at room temperature.

Methods for freeze-drying of foods have had numerous applications and have great potential for the preservation of many different types of biological samples (Werntz, 1967).

Refrigeration

Refrigeration generally implies the storage of biological samples at from +4 to +7°C.

The preservation of whole blood by refrigeration, in the presence of anticoagulants, is a well-known method commonly employed by blood banks (Hurn, 1968). The changes in certain specific blood constituents after refrigeration for varying lengths of time have been studied. Blood platelets from human and other mammalian sources have been of particular interest (Morrison, 1968; Baldini, 1968). However, the survival time of platelets preserved by refrigeration is only eight to ten days, (Baldini, 1968) while the survival time for whole blood is 21 days or longer.

The preservation of whole body organs by refrigeration has come under intensive study (Wolstenholme, 1954; Lillehei, 1964; Blumenstock, 1967; Robertson, 1968). Cooling of organs sharply reduces metabolic activity since oxygen consumption of tissue falls exponentially with temperature reduction. Unfortunately, protection of organs by coöling alone does not exceed five to six hours for most tissues. Beyond this time, irreversible tissue damage occurs. However, a combination of hypothermia at 2° to 4°C and hyperbaric oxygen has allowed whole organ storage up to 72 hours in a viable state (Lillehei, 1964). Several groups of investigators have been able to maintain viable mammalian hearts at subzero hypothermia as long as freezing is prevented (Karow, Jr., 1969). The best results for heart storage have been obtained by perfusion of the organ in an interim host (Humphries, Jr., 1967; Webb, 1969) or by *in vitro* perfusion (Norman, 1968; Childs, 1969; Jacob, 1969; Malinin,

1970). Whole kidney storage has been attempted using one or more of the following methods: (1) hypothermia, (2) hyperbaria, (3) storage in an intermediate host, (4) treatment with metabolic inhibitors, and (5) perfusion with a suitable perfusate (Belzer, 1969).

Human artery preservation by refrigeration for later grafting applications has been used for carotid and other arteries (Dale, 1969; Ross, 1969). In this technique, the artery is removed and immersed in ice water. The muscle and elastic tissue are removed by treatment with ficin. Then the ficin is removed, and the artery is tanned, so to speak, with dialdehyde starch solution. The artery is again washed and tested for leaks before being used as grafting material (Dale, 1969).

Refrigeration of clinical samples such as urine, feces, etc., is covered below under preservation methods applied to mammalian specimens.

Drying

Drying as defined here refers to removal of moisture from a sample that is in any physical state except the frozen state.

One of the more interesting and challenging aspects of drying for preservation of biological materials is "flash drying" (Gordon, 1949). The process involves instantaneous removal of moisture from materials in from 2 to 10 seconds by the application of a turbulent stream of hot air. It is quite common to use air heated to 1300°F for highly inflammable products such as spent grain, fine wood waste, and sewage sludge. Maximum agitation is fundamental, and the smaller the particle the more rapid the moisture removal. Flash drying systems are designed for (1) drying without disintegration, (2) drying with disintegration, or (3) drying and pulverizing (Gordon, 1949).

Evaporation techniques from temperatures of 20° to 100°C are widely used in industrial food processing (Flosdorf, 1959). A variety of equipment concepts for this technique have been explored (Flosdorf, 1959).

The drying of bacteria by cloth drying and spray drying gives a 50% viable recovery of organisms with a storage half-life of greater than 700 days at 40°F (Foster, 1954). Inlet temperatures on the order of 140° to 300°F with collection temperatures varying from 50° to 140°F have been tried. A variety of secondary drying techniques have also been applied such as vacuum drying, package dessication, direct contact with silica gel, and filter cake-air through drying. Regardless of the drying method used, complete removal of water markedly reduces the recovery of viable organisms (Foster, 1954).

Heat drying of foods, or similar materials, is often combined with other processing techniques such as concentration, homogenization, pasteurization, dilution with carrier, blanching, cutting or piercing, or treatment with antioxidants (Morris, 1947; Evans, 1965).

Chemical Preservation

Chemical preservation is used here to mean inhibition of bacterial or fungal multiplication, prevention of cellular change, or preservation of general morphology.

Chemicals for sterilization and preservation of biological materials have been in use for thousands of years. Lawrence and Block (1968) discuss the fundamental and practical aspects of controlling bacteria, protozoa, and helminths by both chemical and physical methods. These authors also include methods for spacecraft sterilization in preparation for a landing on other planets. Several testing methods for measuring the effectiveness of chemical disinfectants and preservatives have been developed. A variety of mammalian body exudates have been sterilized with various chemical treatments. For example, saliva and oral tissues have been sterilized with liquid ethylene oxide or hydrogen peroxide (Williams, 1962). The storage of mammalian waste products in space flight has also been described (Whirlpool Corp., 1964). For this application, solutions of solid germicides such as 8-quinolinol sulfate, sodium orthophenolphenolate mixed with sodium chlorophenolphenolates, and neomycin sulfate plus myrislyl gamma-picolinium chloride were used for stabilizing wet or dry wastes.

The use of antiboitics for preservation of foods such as cheese, fruits, meat, poultry, and fish has been used extensively (Collins, 1967). Salt is still used to preserve food stuffs along with propionates, benzoates (Collins, 1967), and sorbic acid (Luck, 1969).

The use of chemical stains to preserve the morphological detail of specimens for conventional and electron microscopy has been reviewed (Sternberger, 1969). Specific areas of histochemistry have also been extensively studied, such as lipid histochemistry (Adams, 1969). Histochemistry has been developed to include a great many very specific chemical detection reactions for preserving the desired material for later analysis (Lewis, 1962). Plant histology reviews are also available for evaluation of methods for preserving the structural details of plant material for later study (Chamberlain, 1924).

The preservation of biological specimens for gross observation has reached a high state of perfection with the advent of modern plastics. The work of Lutz (1969) summarizes the preservation of vertebrate, invertebrate, and plant specimens by plastic embedding. Various workers have described methods for preservation of zoological specimens (Wagstaffe, 1955; Costello, 1957), invertebrate animals (Galtoff, 1937), botanical specimens (Purvis, 1964), fungi (Sparrow, 1960), and algae (G. Smith, 1950, 1951; Florkin, 1969). Chemical zoology has also been reviewed (Florkin, 1969).

Radiation

There appears to be no doubt that freezing is the current method of choice for the preservation of most biological materials. Radiation, however, may in time become a serious competitor for the preservation of biological materials.

Radiation has been applied mostly to the preservation of food products. Pharmaceuticals have also been sterilized by radiation treatment (Brownell, 1968). Ionizing radiation can penetrate through considerable depth of product, even after packaging, with little rise in temperature and usually little chemical change (Lawrie, 1968). The ions and other activated molecules that the radiation creates are only the first events in a series, and form, for example, free radicals, polymers, and peroxides. With a dose of 5 Mrad (approximately that required for microbial sterility), there is a marked loss of water-holding capacity and change in the behavior of isolated myofibrils in meat, as well as a change in all pH values in the physiological range (Lawrie, 1968). Radiation can eliminate *Salmonella* and various fungi, particularly molds, from any biological product (Lamade, 1968). Either or both electromagnetic or particulate radiation can be used for sterilization (Wilson, 1968). Although complete destruction of microorganisms in food products by radiation is possible, a simple controlling dose of radiation to limit the multiplication of microorganisms is a more likely application (MacQueen, 1969). Very few foods can tolerate the big radiation dose required for total bacterial death, as such a dose produces off taste and odors (Sutton, 1969). Special reference to irradiation of wheat, potatoes, and onions has been made by the World Health Organization (WHO, 1970). Fruits and vegetables have also been extensively irradiated (Holdsworth, 1970).

The Above Techniques Applied to Mammalian Specimens

Special emphasis has been placed on this topic in present and past space flights, and several reviews on this subject are available (Winsten, 1965; Fraser, 1967; Spacelabs, 1967; General Electric, 1967). Below is a general summary of their findings.

Mammalian and related biological samples in the form of gases, liquids, or solids require several different methods of preservation. From the reviews quoted above, it is evident that freezing, lyophilization, refrigeration, drying, collection of gases, and certainly aliquoting of samples into containers containing special chemical preservatives are all required for complete sampling capability.

Specimens that will need to be collected are whole blood, serum or plasma, cerebrospinal fluid, urine, feces, and sweat. Also, specimens from the nasal passage, mouth, throat, and skin may be required. In general, untreated specimens are not acceptable for analysis beyond brief periods of storage, with the exception of inorganic constituents.

Except for metabolic studies, blood specimens should be obtained after an overnight fast or at least four hours after a solid meal. When a substance to be analyzed occurs in blood, serum is usually the preferred sample form. Serum samples should be free of hemolysis and should be separated from the clot within two hours after collection. Heparin, oxalate, citrate, and ethylenediaminetetraacetic acid (EDTA) can keep whole blood from clotting for a number of days. Plasma is required for analysis of animo nitrogen, antihemophilic globulin, fibrinogen, fibrinolytic activity, plasma thromboplastic component, plasma volume, and prothrombin activity. If blood cells, and also spermatozoa, are to be frozen, cryoprotectants--such as glycerol, DMSO, PVP, or dextran--are required to prevent injury from freezing. Even with these additives, white blood cells and platelets are, for the most part, destroyed without special handling techniques. There is no single method that can be used to preserve the different cellular components in whole blood, but rather each component should be separately isolated, and then each preserved individually according to its own characteristics to minimize destruction.

Hematocrit; karyotyping; platelet adhesiveness; platelet, red blood cell, and reticulocyte counting; clotting time; clot retraction; white blood cell differentials; white blood cell motility; and phagocytic activity cannot be measured on whole blood that has been frozen. Blood smears made from fresh whole blood treated with anticoagulant can be used for reticulocyte counting, white blood cell differentials, and a rough platelet count. The measurement of blood pH, pCO_2, pO_2, ketone bodies, clotting time, bleeding time, number of blood cells, and clot retraction appears to require real-time analysis. However, gas samples can be stored in glass or metal containers for later analysis, and blood smears can be made for counting many of the blood cells at a later time. Whole blood absorption on paper for later analyses of hemoglobin, alkaline phosphatase, glucose, phenylalanine, cholinesterase, uric acid, urea, ketone bodies, bile pigments, adrenalin, peptides, and nucleotides has been tried, and the method appears to have long term storage capability at room temperature (Comstock, 1966; Rice, 1967). Antibiotics have been used to prevent bacterial growth in blood (Bayliss, 1954). Lyophilization and spray drying have been used to preserve plasma and serum.

All organic chemical assays should be performed within five hours after sample collection if possible. If this cannot be done, then the sample should be refrigerated between 2^0 and 4^0C. If the dalay is greater than 24 hours, the sample is best preserved at a minimum of -12^0C. Most substances of this type, when frozen, are well preserved with the exception of some enzymes and proteins. Freezing and thawing denatures some proteins and lowers the concentration of other constituents. Thawing should usually be done rapidly in a 37^0 or 45^0 water bath.

Urine should be refrigerated during collection with the required preservative present in the collection container. Slight acidification of urine, usually with boric or benzoic acid, is necessary to preserve

the structures of the 17-hydroxy steroids, serotonin, and 5-hydroxyindoleacetic acid, while stronger acidification by compounds such as HCl or potassium bisulfate is required to preserve the catecholamines and antidiuretic hormone. Chloroform, formaldehyde, toluene, phenol, and thymol have been used as urine preservatives to retard bacterial growth. Freezing to -20^{o}C with acid preservatives can meet urine storage requirements for at least two months. Lyophilization has also been applied successfully to the preservation of urine.

Sweat and feces can be preserved for four to seven days by refrigeration for later analysis of most of the organic compounds found in these biological materials. However, freezing to -100^{o}C without exposure to light is the long term preservation technique of choice. Lyophilization can also be used in some cases.

III. TABLES OF TECHNIQUES OF PRESERVATION

ABBREVIATIONS

abs - absolute

ACD - acid-citrate-dextrose anticoagulant

alc - alcohol

alk - alkaline

amt - amount(s)

app - apparatus

approx - approximately

aq - aqueous

atm - atmosphere(s)

ATP - adenosine triphosphate

ATPase - adenosine triphosphatase

av - average

biol - biological

^{o}C - degrees Centigrade

cc - cubic centimeter(s)

chem - chemical

cm - centimeter(s)

compd - compound(s)

conc - concentration

contg- containing

CPD - citrate-phosphate-dextrose anticoagulant

detd - determined

detn - determination

diam - diameter

distd - distilled

DMSO - dimethyl sulfoxide

DNA - deoxyribonucleic acid

DPG - diphosphoglycerate

EDTA - ethylenediaminetetraacetic acid

exptl - experimental

ext - extract(s)

extd - extracted

extn - extraction

^{o}F - degrees Fahrenheit

ft - foot/feet

gm - gram(s)

Hb - hemoglobin

HCl - hydrochloric acid

hr - hour(s)

l - liter(s)

liq - liquid

max - maximum

M - molar

mag - magnetic

mg - milligram(s)

min - minute(s)

mixt - mixture(s)

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ml - milliliter(s)

mm - millimeter(s)

mM - millimole(s)

mon - month(s)

mOsm - milliosmol(s)

Na_2EDTA - disodium salt of ethylene-diaminetetraacetic acid

NaOH - sodium hydroxide

no. - number

org - organic

pH - hydrogen ion concentration

phys - physical

physiol - physiological

physiol saline soln - physiological saline solution

powd - powder

prepn - preparation(s)

PVC - polyvinyl chloride

PVP - polyvinylpyrrolidone

satd - saturated

sec - second

sol - soluble

soln - solution(s)

temp - temperature(s)

TPN - triphosphopyridine nucleotide

UV - ultraviolet light

vol - volume(s)

v/v - volume by volume

wt - weight

w/v - weight by volume

yr - year(s)

> - greater than

< - less than

μ - micron

μgm - microgram(s)

μl - microliter(s)

% - percent

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TABLES

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FREEZING

SPECIMEN DESCRIPTION	ORIGINAL FORM OF SAMPLE	BRIEF DESCRIPTION OF METHOD	LIMITATIONS OF METHOD	OTHER COMMENTS ABOUT METHOD	ESTIMATED PRESERVATION TIME	SURVIVAL TIME	NO. OF STEPS	EQUIPMENT AND REAGENTS USED	END USE OF SPECIMEN	REFERENCE
1. Adrenal glands	Pellet-2 homogenate of rat adrenal glands	Rat adrenal tissue was preincubated, and pellet-2 was isolated from KCl homogenates and resuspended. The pellet-2 prepn fortified with TPN, glucose-6-phosphate, and glucose-6-phosphate dehydrogenase was analyzed for pregnenolone synthesis in the presence of Ca^{2+}, Ba^{2+}, or Sr^{2+}, with or without freezing.	Pregnenolone synthesis was stimulated by Ca^{2+}. Ba^{2+} or Sr^{2+} at higher conc could replace Ca^{2+}, and stimulation was not due to pro-enzyme activity. At pH 6.2 freezing pellet-2 decreased pregnenolone synthesis. At pH 7.5 the frozen pellet was more active than the normal one. Reasons for these phenomena were discussed.			Not given	Not Clear	Incubator, freezer, containers 0.154 M KCl, TPN, glucose-6-phosphate, glucose-6-phosphate dehydrogenase, EDTA, phosphite buffer, bicarbonate buffer, Ca^{2+}, Ba^{2+}, Sr^{2+}	Effects of freezing on enzyme activity	Koritz (1962)
2. Algae	Algal cultures	Algal cells to be frozen were harvested from vigorous cultures 4-8 days after inoculation and were suspended in a 10% glycerol soln. Samples of the suspension were placed in sterile vials and plugged with cotton. Then they were frozen either by controlled or uncontrolled heating to temp down to -196°C. The effects of freezing techniques on cell viability were observed.	Controlled cooling at 1°C/min to -196°C in 10% glycerol gave more intact, viable cells and more mitotic activity than uncontrolled cooling.	Euglena strains reacted the same as other algal strains to freezing and thawing, except there was a delay in the motility of cells in the recovered cultures.	1-2 hr	19-36 mon	3-4	Sterile vials, cotton plugs 10% glycerol in demineralized water	Maintenance of type cultures	Hwang (1965)
3. Arteries, corneas and adrenal glands	Human arteries, corneas, and adrenal glands from cadavers	Human arteries were taken from cadavers dead less than 12 hr that had been placed in cold storage at least 2 hr. The arteries were placed in sterile isotonic saline. Then they were placed in sterile pyrex tubes and frozen in a mixt of dry ice and alc at -79°C for 5 min. Afterward they were stored at -70°C or less. A similar technique was used for adrenal glands and cornea except a glycerol-saline soln was used.	Arteries were maintained in a tissue bank up to 166 days, but showed no growth; adrenals were kept 6 mon but, as they were intended for autografts, were never used; corneal grafts remained clear up to 4 wk.		Not given; > 1 hr?	Arteries, 160 days; adrenals, 6 mon; corneas, 4 wk	3-5	Freezer, test tubes, surgical tools, alc-dry ice bath Saline soln, glycerol-saline soln, alc-dry ice mixt	Transplants	Rob (1954)

FREEZING (Continued)

SPECIMEN DESCRIPTION	ORIGINAL FORM OF SAMPLE	BRIEF DESCRIPTION OF METHOD	LIMITATIONS OF METHOD	OTHER COMMENTS ABOUT METHOD	ESTIMATED PRESERVATION TIME	SURVIVAL TIME	NO. OF STEPS	EQUIPMENT AND REAGENTS USED	END USE OF SPECIMEN	REFERENCE
4. Beetles, Carabid	Whole live carabid beetles	Carabid beetles were collected at various intervals over a yr, and were maintained at the temp found until used. They were then fitted with a thermocouple affixed to the dorsal abdomen with wax, and were placed in insulated vials in a regulated bath. The temp was lowered to various levels, the beetles were thawed to room temp, and the results observed.	Winter beetles tolerate temp below -35°C but summer beetles die if frozen to -6.6°C. Winter beetles freeze at about -10°C and thaw near -3.5°C. Summer beetles thaw at -0.7°C. Cooling rates must be near 20°C/hr or less to avoid freezing damage.	Beetles had to be capable of walking, feeding, and avoidance response, with no paralysis or erratic behavior noted for up to 4 days, for them to be considered 'survived'. Winter beetles apparently adapt and can withstand much lower temp than summer beetles. This may be due to changes in hemolymph glycerol.	<1 hr	Up to 8 hr	2	Constant temp bath, copper constantan thermocouple, wax, insulated vials, drying oven	Study of freezing effects on adult insects	Miller (1969)
5. Bone marrow	Fresh marrow from needle puncture of the human iliac bone	Bone marrow obtained by needle puncture of the iliac bone was aspirated and transferred to a collection bottle. Then it was filtered, and an eq vol of suspending fluid contg DMSO or glycerine and TC 199 culture fluid was added. The mixt was centrifuged, and the marrow cells added to polythene aluminum bags. The bags were heat sealed and frozen at -1°C/min to -15°C, then to -196°C.	25% of the cells were viable after 2 yr storage at -79°C.	The cryoprotective agent was not washed out before transfusion.	1-2 hr	2 yr at -79°C with 25% cell survival	8-9	Heparinized hypodermic syringe, collection bottles, centrifuge, Millipore filter, polythene aluminum bags, heat sealer, CO_2 or liq nitrogen freezer Heparin, 15% glycerol, TC 199 tissue culture fluid, DMSO	Bone marrow transplantation	Pegg (1964)
6. Bone marrow	Fresh bone marrow from rat or mouse femurs	Bone marrow cells obtained from the femurs of mice or rats were suspended in Hank's soln, strained through a fine mesh, resuspended in Hank's soln, and finally resuspended in freezing media contg 15-25% PVP K-15 and K-17, 10% PVP K-30, and 10% DMSO in Hank's soln contg 30% rat or mouse serum. 2 ml aliquots were slowly frozen to -25°C in ampules, and finally to -196°C. Various tests were carried out to evaluate the cryoprotective effectiveness of PVP.	The optimum conc of PVP K-15 and K-17 was 20%. Low mol wt PVP was comparable to DMSO in cryoprotection, but was more desirable because of its rapid renal excretion.	PVP samples, which were acidic in nature, were neutralized before use.	2-3 hr	Up to 6 mon	6-7	Fine mesh strainer, flasks, ampules, Dewar flask, copper coil, thermocouple, recording galvanometer Hank's soln, PVP K-15 soln, PVP K-17 soln, PVP K-30 soln, DMSO, liq nitrogen, alc	Bone marrow transplants	Persidsky (1965)

FREEZING (Continued)

SPECIMEN DESCRIPTION	ORIGINAL FORM OF SAMPLE	BRIEF DESCRIPTION OF METHOD	LIMITATIONS OF METHOD	OTHER COMMENTS ABOUT METHOD	ESTIMATED PRESERVATION TIME	SURVIVAL TIME	NO. OF STEPS	EQUIPMENT AND REAGENTS USED	END USE OF SPECIMEN	REFERENCE
7. Bone marrow	Fresh aseptically collected human and mouse bone marrow	Aseptically collected human and mouse bone marrow were mixed with ½ vol of 30% glycerol or 10% DMSO in Osgood medium. Samples were frozen in vials in a freezer and maintained at -85°C.	Morphological preservation was good in human bone marrow frozen for 7 yr. Prolonged storage resulted in less marrow cells being capable of excluding Trypan Blue. The capacity to incorporate thymidine was well preserved during prolonged storage.	Samples were thawed by immersion in an ice water bath. To each tube of thawed bone marrow suspension ½ vol of 33 1/3% cold glucose soln was added.	< 1 hr	7 yr	3-4	Surgical tools, hypodermic needles, siliconized syringes, screw-cap tubes, Canalco Freezer (Model 60 RC), Harris freezer 15% glycerol in Osgood's medium, heparin, 10% DMSO in Osgood's medium	Reinfusion for radiation protection	Kurnick (1967)
8. Bone marrow	Freshly removed canine bone marrow	Allogeneic canine marrow, preserved in 70% TC 199 medium (Difco), 20% DMSO, and 10% autologous serum, was frozen in plastic bags between copper plates to -80°C. The stored marrow was used for transplantation in unrelated dogs following 1200 R whole body irradiation. Histocompatibility of matched and mismatched recipients were compared.	Matched recipients survived longer than mismatched recipients. Two matched dogs were alive more than 135 and 160 days respectively after transplantation. Freezing does not diminish the ability of marrow to produce the lethal graft-host disease.		1-1½ hr	Up to 47 days	5-6	Surgical tools, stainless steel screens, flasks, plastic blood bags, copper plates, cobalt-60 source TC 199 medium (Difco), 10% autologous serum contg 6.6 mg heparin, 20% DMSO, methotrexate, sodium ampicillin	Transplants	Epstein (1969)
9. Bone marrow	Freshly excised mouse bone marrow	Mouse femur bone marrow cells were suspended in Hank's soln contg 4% calf's serum plus either 12% glycerol or 12% DMSO at a conc of 90,000 cells/ml. The suspension was frozen in ampules to -100°C at 2°C/min.	Hematopoietic stem cells preserved in either glycerol or DMSO at -100°C replenish themselves at the same rate as stem cells from fresh marrow.	Glycerol was removed by serial dilution with 35% glucose and 6% dextran.	1 hr	Ampules were thawed immediately	4-5	Ampules, containers, Linde BF-3 liq nitrogen freezer, water bath Hank's soln, 4% calf's serum, 12% glycerol, 12% DMSO	Marrow transplantation	O'Grady (1969)
10. Bone marrow	Fresh human and dog marrow	Human and dog bone marrow were collected by aspiration from the iliac crest and diluted with TC 199 medium contg heparin. The cell suspensions were further diluted with TC 199 contg either 30% glycerol or DMSO. The samples were sealed in 25 ml ampules and cooled at 1°C/min to -79°C for glycerol-protected cells and to -196°C for DMSO-protected cells. The results were compared after 3 yr of storage.	Bone marrow frozen with glycerol to -79°C was almost entirely destroyed after 3 yr, while marrow frozen with DMSO at -196°C was largely intact after 3 yr.	Glycerol was removed postthaw by gentle centrifugation.	1-2 hr	Up to 3 hr	5-6	Hypodermic syringe, polyethylene ampules, carbon dioxide or liq nitrogen freezer Glycerol, DMSO, TC 199, heparin	Transplants	Malinin (1970)

FREEZING (Continued)

SPECIMEN DESCRIPTION	ORIGINAL FORM OF SAMPLE	BRIEF DESCRIPTION OF METHOD	LIMITATIONS OF METHOD	OTHER COMMENTS ABOUT METHOD	ESTIMATED PRESERVATION TIME	SURVIVAL TIME	NO. OF STEPS	EQUIPMENT AND REAGENTS USED	END USE OF SPECIMEN	REFERENCE
11. Borrelia anserina	Whole blood infected with Borrelia anserina	Fowl blood contg Borrelia anserina was either diluted with an eq vol of sterile 1% citrate saline or allowed to clot. The citrated sample was refrigerated for a couple of hr, while the clotted sample was held at room temp and the serum removed. The serum and a portion of the citrated blood were mixed with an eq part of 15% glycerol, placed in an ampule inside a larger ampule, and held at -30°C for 20 min. Next the ampules were held over liq nitrogen vapor and finally stored in liq nitrogen.	Borrelia anserina remained viable for 150 days in fowl serum and citrated blood, with or without glycerol, when stored in liq nitrogen.		36-37 hr	150 days	6-7	Hypodermic syringe, refrigerator, containers, ampules, deep-freeze, liq nitrogen freezer 1.0% citrate saline soln, 15.0% glycerol soln	Maintenance of cultures for production of fowl tick fever vaccine	Hart (1970)
12. Borrelia kansas and Plasmodium berghei	Infected mouse blood	Albany white mice were infected with Borrelia kansas or Plasmodium berghei. On the 2nd day after infection with Borrelia and the 7th with malaria, blood was removed for storage. One part blood was added to 25 parts sterile Thioglycollate Medium (Difco) contg 10% glycerol, and the mixt was added to 1 or 5 ml ampules which were heat sealed and slow-frozen to either -55°C or -195°C.	75-80% of Borrelia organisms remained actively motile after 6 mon, and blood cells remained in good condition. Some hemolysis occurred with malaria-infected blood, but both organisms exhibited little change in rate or degree of parasitemia over a 6 mon period. No difference in results was observed at either temp.	Many intracellular malarial parasites exhibited amoeboid movement.	2 hr (Not including inoculation procedure)	6 mon-2 yr	5	White mice, hypodermic syringes, ampules, heat sealer, liq nitrogen freezer, dry ice freezer Thioglycollate Medium (Difco) contg 10% glycerol	Maintenance of viable parasitic organisms with constant virulence, antigenicity, and related properties	Allen (1970)
13. Brain	Whole nephrectomized rat	Live rats were guillotined so that the heads fell into 20 vol of liq nitrogen while the torso blood drained into a dish contg heparin. The cerebral hemispheres, cerebellum, and brain stem were removed with bone clippers in a -20°C room and ground in a mortar under liq nitrogen. The brain powd was weighed and homogenized with 6 M $HClO_4$. Homogenates were removed to a 4°C room, diluted with 1.22 mM EDTA, and centrifuged. Supernatants were neutralized and stored at -70°C until used for enzyme studies on uremic rats.	Very little information was given about the limitations of the preservation method. Rat brains held in an ischemic state for 30 sec before freezing were compared to rat brains frozen immediately for levels of several enzymes. The levels varied. It was implied that rat brains frozen immediately retain most enzyme systems as they were at the time of decapitation.		Approx 1 hr	Not given	7-8	Rat guillotine, liq nitrogen container, bone clippers, cold room (-20°C), mortar and pestle, homogenizer, cold room (4°C), centrifuge, liq nitrogen freezer, other containers, balance 6 M $HClO_4$, 1.22 mM EDTA	Brain metabolism studies in uremic and adenosine-infused rats	Van den Noort (1968)

FREEZING (Continued)

SPECIMEN DESCRIPTION	ORIGINAL FORM OF SAMPLE	BRIEF DESCRIPTION OF METHOD	LIMITATIONS OF METHOD	OTHER COMMENTS ABOUT METHOD	ESTIMATED PRESERVATION TIME	SURVIVAL TIME	NO. OF STEPS	EQUIPMENT AND REAGENTS USED	END USE OF SPECIMEN	REFERENCE
14. Brain	Freshly excised mouse brain	Mice were decapitated and their brains removed within 30 sec and dropped into petroleum ether cooled to -70°C in a dry ice-ethanol bath. Brains were mounted in the frozen state on a cryostat and sectioned at -20°C. Five to six sections were dropped into cooled test tubes, and the tubes were briefly handheld to melt the sections to the bottom. The tubes were immediately returned to -20°C until used.	Brain sections stored in this manner were suitable for histochem enzyme studies especially gamma-amino butyrate transaminase.		Few min	Not given	6-7	Surgical tools, dry ice-alc bath, cryostat, test tubes Dry ice, alc, petroleum ether	Histochem enzyme studies	Van Gelder (1968)
15. Brain	Whole live rats	Rats were killed by immersion in liq nitrogen, and the whole brain was removed and weighed. The brain was then pulverized in a 50 ml steel centrifuge tube embedded in solid CO_2. The tube was then placed in an ice bath, and 0.3 M cold $HClO_4$ soln was pipetted into the tube to give a final vol of 10 ml brain ext. This was homogenized and centrifuged at 0°C. The supernatant was filtered at 4°C into a test tube and was ready for anal of the adenine nucleotides.	ADP conc was higher using this method than it was after extn with trichloroacetic acid in acetone at -78°C. ATP and AMP were the same using either method. ATP conc was higher in freeze-dried brains than it was in frozen ones. AMP, ADP, or total nucleotides were the same for both methods. Brain extn using this method satisfactorily prevents breakdown of brain ATP.	The Kalckar method of analyzing nucleotides in brain ext was unsuitable because AMP was too high.	½ hr	Not given	10-11	Liq nitrogen bath, steel centrifuge tube, ice bath, pipette, plastic pestle, homogenizer, centrifuge, tubes, refrigerator Dry ice, liq nitrogen, cold 0.3 M $HClO_4$	Assay of rat brain adenine nucleotides	Wilson (1969)
16. Bacteria	Organisms suspended in aq soln	Aerobacter aerogenes was protected from freezing damage by most of the compd that protect red blood cells. 18 aq soln (usually 10% soln) were tested on 95-99% viable organisms, which were then fast frozen on liq nitrogen. DMSO, human albumin, and ovalbumin gave essentially complete protection (95-98% viability) as compared to 50% viability with unprotected controls.	Several protective agents gave 95-98% viability after rapid freezing and thawing. The others gave less protection or none at all.	Non-penetrating polymeric solutes gave the best protection.	Few min	Not given	3-4	Test tubes, liq nitrogen freezer, pipettes, reagent bottles Dimethyl sulphoxide, dimethyl acetamide, dimethyl formamide, N-methyl-2-pyrrolidone, formamide, 2-pyrrolidone, tetrahydrofuran, acetone, pyridine N-oxide, butyrolactone, acetonitrile, polyethylene glycol, polyvinyl pyrrolidone, human albumin, ovalbumin, acetamide, urea, liq nitrogen	Study of the effectiveness of cryoprotectants on bacteria and blood	Nash (1963)

FREEZING (Continued)

SPECIMEN DESCRIPTION	ORIGINAL FORM OF SAMPLE	BRIEF DESCRIPTION OF METHOD	LIMITATIONS OF METHOD	OTHER COMMENTS ABOUT METHOD	ESTIMATED PRESERVATION TIME	SURVIVAL TIME	NO. OF STEPS	EQUIPMENT AND REAGENTS USED	END USE OF SPECIMEN	REFERENCE
17. Bacteria	Bacterial cultures	Pseudomonas 10H and Escherichia coli try-(tryptophane-requiring mutant) were suspended in physiol saline and Sørensen phosphate buffer respectively. Ampules contg samples of the suspension were cooled and thawed at various rates, and the effects on genetic stability of the bacteria were investigated. Some samples were cooled and thawed several times, and the survivors were counted and tested for sensitivity to antibiotics and sulfonamides.	No change in response of Pseudomonas to freezing and thawing damage or to drugs was observed over 18 cycles of treatment. Freezing at various temp between 0° and $-196^{\circ}C$ did not produce mutants in Escherichia coli try^{-}, therefore it was concluded that freezing and thawing are not mutagenic to bacteria.		Varied	Not given	4-6	Culture dishes, nutrient agar plates, incubator, ampules, Linde BF3 freezer Difco Bacto Unidisks, physiol saline, Sørensen phosphate buffer, liq nitrogen	Mutation studies	Ashwood-Smith (1965)
18. Bacteria	Bacterial suspension	Streptomyces viridoflavus shake flask cultures were subdivided into sterile, cotton-plugged 1 ml ampules and stored in vapor phase canisters in a liq nitrogen refrigerator. Eq vol of 20% glycerol were added to some samples. Tables for Candidin production by cultures grown from slants after storage and transfer were given. Other organisms were tested in liq nitrogen also.	Both slants and mycelial suspensions of S. viridoflavus were weakly viable, but fully productive after 1 yr at $-20^{\circ}C$. Viable counts were constant at $-185^{\circ}C$ over a 12 mon storage period. Escherichia coli B suspensions showed no decreases in count after storage in liq nitrogen for 15 mon.	Liq nitrogen storage has great potential value for preserving culture samples for future examination without subculturing.	Few min	Up to 1 yr	3	Linde L-35-9 liq nitrogen refrigerator, glass ampules, cotton stoppers, flask shaker, flasks, agar plates, cold canisters Soybean meal-glucose medium, yeast ext-glucose medium, liq nitrogen	Culture maintenance	McDaniel (1968)
19. Bacteria	Cultured cells or bacteria	Bacteria that grow well in either an ordinary infusion broth or standard thioglycolate broth were grown, scraped off, emulsified in broth, then sealed in 0.7 Cryules (containers). The Cryules were placed in a hollow aluminum cane and immediately immersed in liq nitrogen. Canes and ampules can be marked for easy retrieval.	No organism or tissue culture tried failed to grow after being stored by this method. (Organisms were not listed).	Only young, actively growing cultures were used. Some organisms were killed during fast freezing, but the authors considered the convenience more important than the loss.	Few min	The authors felt frozen samples would last indefinitely	4-5	Cryules (Wheaton glass) aluminum cane, Linde freezer Liq nitrogen, infusion broth, thioglycolate	Type cultures or cell-line preservation	Marymount (1969)

FREEZING (Continued)

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20. Bacteria	Cell suspensions	44 marine and 5 nonmarine strains of bacteria were freeze-dried in 1958 and then stored at room temp. Other samples were suspended in glycerol-nutrient broth and stored at -29°C. Total viable counts were made periodically for 10 yr, and the two methods of preservation were compared.	After 10 yr 9% of the freeze-dried cultures and 41% of the frozen cultures were nonviable. Corynebacteria and micrococci withstood preservation the best, while vibrios and photobacteria were the least viable after storage.	Gram positive organisms tend to survive longer periods of storage than Gram negative organisms.	Not given; depends on method	Up to 10 yr	Varies with method	Lyophilizer, ampules, freezer, heat sealer Glycerol, appropriate culture media	Maintenance of type culture	Greig (1970)
21. Blood	Freshly collected human blood	Twelve units of ACD-collected fresh blood were centrifuged at 10°C to concentrate the red cells. One unit of packed cells was stored at 4°C, and the remaining 11 were frozen to -120°C by the low glycerol-sucrose method of Pert (1967). Another set of 12 units was frozen at -170° C. After storage and thawing, hemolysates were prepared and enzyme assays were run on these hemolysates.	After 19 wk storage at -120°C or -170°C the activity of red blood cell enzymes was essentially unchanged, whereas several red blood cell enzymes were reduced in activity by ½ to 1/3 when stored at 4°C.		1 hr	Up to 19 wk	3-5	ACD double plastic packs, centrifuge, plastic bags, freezer Glycerol, sucrose, ACD soln	Tranfusions	Mourad (1965)
22. Blood	Freshly drawn human blood	Blood from healthy donors was collected into standard ACD anticoagulant. Within 5 days of collection the blood was centrifuged. An eq vol of 5-5.6 M glycerol soln, also contg glucose, fructose, and Na_2EDTA, was added to the packed red cells in a sterile, disposable blood-freezing unit. The units were then frozen to -85°C.	90±5% of red blood cells could be recovered from whole blood using this method. 3% of the loss was hemolytic, the rest mechanical. 87-95% of treated red blood cells circulated 24 hr after transfusion with the slope of decline after 24 hr slightly longer than usual. Blood stored 2 yr shows no appreciable breakdown, and storage life may approach 10 yr.	Free Hb was less than 50 mg for resuspended cells reconcentrated to a hematocrit of 80 or 90%. This was less than 21 day ACD blood stored in glass bottles. Donor's plasma protein and isoagglutinins of Group O blood were eliminated by washing after thawing. Also hepatitis seemed to be removed by washing.	7 hr (including complete freezing)	2-10 yr	4-5	Blood collection app, IEC Huggins cytoglomerator, disposable plastic blood-freezing units, Harris twin dual cascade freezer 5.6 M glycerol soln contg also glucose, fructose, and Na_2EDTA; 50% glucose soln; 8% glucose and 1% fructose soln; physiol saline	Transfusions	Huggins (1966)

FREEZING (Continued)

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23. Blood	Freshly collected whole human blood	A simple blood processing and storage procedure was described for routine use which will provide a continuous daily supply of whole blood tranfusions with coagulation factors similar to fresh blood. The method also provides fresh frozen plasma, packed cells, and plasma for fractionation. Blood can be collected on a routine basis.	Packed cells were kept 21 days and plasma 1 yr. The author felt they could be preserved longer.	The technique involved separating the plasma and packed cells initially and recombining them as needed. 50 units of blood can be stored in a freezer at one time.	< 30 min	12 mon for plasma; 21 days for whole blood	4-5	Specially designed refrigerator-freezer (described), refrigerated centrifuge, blood collection app, plastic bags, clamps, tubing ACD anticoagulant	Transfusions	Allen (1967)
24. Blood	Freshly drawn human blood	Blood units were prepared with a final conc of 15% hydroxyethyl starch as the protective material. The units were frozen in metal containers in liq nitrogen at -196°C. During freezing the blood mixt was agitated at 200 cycles/sec to assure proper mixing.	Av recovery of red blood cells in vitro was 97.4%, saline stability averaged 83.4%, and Hb in the plasma averaged 283.3 mg/100 ml.	Hydroxyethyl starch has cryophylactic properties similar to PVP. It has the added advantage of being metabolized by the recipient which eliminates the need for extensive processing prior to transfusion.	<1 hr	1 wk	4-5	Metal containers, agitator, Linde blood-processing app, liq nitrogen container Hydroxyethyl starch	Transfusions	Knorpp (1967)
25. Blood	Fresh whole human blood	Several whole blood samples were collected in ACD soln from patients at 2 or 4 wk intervals preceding surgery. The blood was stored at 4°C for up to 5 days. Then each unit was centrifuged in a refrigerated centrifuge, and the plasma was removed and stored at -20°C. Packed red cell mass was transferred to a blood-freezing unit and glycerolized with an eq vol of 8.6 M glycerol soln contg 0.3% Na_2EDTA, 8% glucose, and 1% fructose. The red cell mass was then slowly frozen to -80°C and stored for 2 mon. The red cell mass was thawed, recombined with autologous plasma, and used during surgery on its donor.	Blood collected at 4 wk intervals and stored up to 2 mon was used during surgery on its donor without serious side effects. No significant decrease in peripheral hematocrit level was noted.	Use of this method eliminated blood transfusion complications during elective surgery.	Not given; approx 1 hr	Up to 2 mon	8-10	Blood collection app, refrigerated centrifuge, blood-freezing unit (Int Equip Co), Huggins Cytoglomerator, glass containers 50% aq dextrose soln ACD medium (NIH formula A)	Autotransfusion during surgery	Daane (1969)

FREEZING (Continued)

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26. Blood	Fresh blood from healthy donors	Blood from healthy donors was collected into ACD anticoagulant. Within 6 days of collection the blood was centrifuged, plasma was removed, and cells were transferred to the lower end of the blood freezing unit. An eq vol of 8.6 M glycerol soln was added to the packed cells with continuous mixing with a mag stirrer. The blood-freezing unit was removed from the cytoglomerator. The package was then laid horizontally in the -85°C freezer. Separately stored aliquots were used for compatibility testing.	Blood was still viable for transfusions after 4 yr of storage.	Cells preserved in this manner were easily transported in dry ice thus permitting international exchange of rare blood types. Many serious side-reactions of blood transfusions were eliminated, including serum hepatitis.	Not given; < 1 hr	> 4 yr	5-6	Blood-freezing unit, donor collection app, freezer, cytoglomerator, sterile spiked coupler, pipettes, mag stirrer, water bath, centrifuge 8.6 M glycerol soln, 5% fructose soln, 50% glucose soln, ACD anticoagulant	Blood transfusions	Huggins (1969)
27. Blood	Whole blood, blood cells, or serum from hibernating ground squirrels	Whole blood, washed cells, or serum from hibernating ground squirrels and woodchucks was stored in liq nitrogen for 4-5 mon. The blood was used to show that a 'trigger factor' for inducing summer hibernation in nonhibernating hibernators could be preserved cryogenically <u>in vitro</u>.	'Trigger factor' for causing summer hibernation was still active after 4-5 mon storage at liq nitrogen temp.	Washed cells, whole blood, and serum all caused nonhibernating hibernators to hibernate, and the reaction does not appear to be species specific.	Not given; few min	4-5 mon or longer	1-2	Liq nitrogen freezer, containers for blood, centrifuge	To trigger hibernation in nonhibernating hibernators	Dawe (1970)
28. Blood, kidneys and livers	Human blood cells in lactose-physiol saline soln; whole rabbit organs	Human blood in lactose-physiol saline soln in an aluminum container coated with vaseline was frozen in liq nitrogen. Whole rabbit kidneys and livers were dipped in glycerol and frozen by immersion in liq nitrogen. In both cases the thermally-insulating coating increased the cooling rate and cell viability. The thermodynamics of this were discussed.	Red cell recovery was 84% on thawing in 40°C water. Viable cells increased by 30% in the coated whole organs to a total of 89% viable cells.	Heat transfer by means of insulating coatings was applicable only in boiling liq.	5 min	Not given	2-4	Nitrogen container, rectangular aluminum container, dipping containers Glycerol, vaseline, lactose-physiol saline soln	Transplants	Cowley (1961)

FREEZING (Continued)

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29. Blood cells, Red and Sperm	Freshly drawn human and bovine red blood cells or fresh bull ejaculate	Human or bovine red blood cells were suspended in 0.16 M sodium chloride soln contg 0.1% glucose, then cooled to 0°C. Glycerol or DMSO was added to 2, 5, 10, or 15% by wt. After allowing intervals of from 10 sec-2 hr for the solute to penetrate the cells, samples were frozen to -79°C by immersing tubes contg the samples in a bath of alc and dry ice. After 15 min the samples were thawed, and the extent of hemolysis was observed. Bull sperm was frozen under similar circumstances to -30° and -79°C.	30 sec of prefreezing equilibration with DMSO was sufficient for complete protection of red blood cells from hemolysis during freezing. Glycerol was more effective for protecting sperm, although DMSO did not seem to be particularly toxic to sperm.	DMSO was recommended where permeability of membranes to glycerol was slight.	15 min	Not given	4	Cooling bath with alc and dry ice, tubes, pipettes, water bath, centrifuge Glucose, sodium chloride, dry ice, ethanol, glycerol, egg yolk-citrate diluent, DMSO	Studies of freezing phenomena in cells	Lovelock (1959)
30. Blood cells, Red	Freshly drawn human blood	Washed, human red cells were suspended in buffered physiol saline soln contg different amt of pyridine N-oxide, and frozen 15 min at various temp between -20° and -80°C. They were thawed, centrifuged, and analyzed for the amt of hemolysis in the supernatant.	A sharp rise in hemolysis occurred at -40°C, and, although limited protection was found, pyridine N-oxide was both penetrating and physically non-toxic up to high conc.		Few min	Not given	3	Blood collecting app, centrifuge, freezer Buffered physiol saline, pyridine N-oxide	Cryoprotectant for red blood cells	Nash (1961)
31. Blood cells, Red	Freshly collected whole human blood	Whole blood was treated with 1 mg EDTA/ml blood. Not longer than 2 hr before freezing, the blood was mixed with half its vol of 40% aq sucrose soln (domestic granulated sugar can be used). The blood was frozen by dripping it into liq nitrogen where it formed frozen pellets 2-3 mm in diameter. The rate of freezing was approx 1-2 ml blood/min. Pellets were removed from liq nitrogen by decanting, placed in cardboard boxes filled with liq nitrogen, and stored in a liq nitrogen freezer.	More than 90% of the red blood cells were recovered intact immediately after freezing. This recovery rate was maintained over 1 yr.	Anticoagulated blood samples could be stored one to two days before freezing. Although certain agglutinogens deteriorated in avidity and titration scores during the yr, preservation was as good if not better than that generally seen after storage in glycerol.	1-2 ml/min	1 yr	4-5	Liq nitrogen freezer, liq nitrogen collection container, funnel, hypodermic needle, tygon tubing, retort stand, cardboard storage boxes, glass containers Liq nitrogen, EDTA, 40% aq sucrose soln	Red cell antigen standards for identification of blood group antibodies	Huntsman (1962)

FREEZING (Continued)

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32. Blood cells, Red	Freshly drawn blood	Red blood cells were frozen rapidly by dropping sealed polythene-coated aluminum foil packets into liq nitrogen. The cells were protected by sugars (glucose, lactose and/or sucrose) or by 10% PVP. Use of PVP allows blood cells to be used for transfusion.	A 97% postthaw recovery was reported for red cells frozen and thawed in aluminum-polythene packets with 10% PVP as an additive. Red cells should remain viable for several mon if the temp is maintained.	PVP as a preservative allowed the blood sample to be used for transfusions whereas high conc of sugar (5% glucose plus 9.35% sucrose) were not suitable for transfusions.	10-15 min	Indefinite	3	Liq nitrogen container, foil-polythene packets, wire rack to packets PVP, glucose, lactose, sucrose	Study of the effects of various additives on blood freezing	Greaves (1963)
33. Blood cells, Red	Freshly collected human blood	10 cc aliquots of chromium-labeled, autologous red cell, which had been preserved with glycerol by the slow-freeze technique and washed with a nonelectrolyte sugar soln by the agglomeration technique, were transfused into healthy recipients. Clinical evaluation of the performance of such samples <u>in vivo</u> was given.	Red cells had acceptable survival rates after 7½ mon at -80°C, but cells stored at -20°C did not. Thawed erythrocytes that had been washed and disaggregated with isotonic saline could be stored postthaw for 6 days at 4°C in saline and 5-9 days in autologous plasma at a hematocrit of 45%. Saline is preferred over autologous plasma for postthaw washing.	Addition of EDTA to the glycerolizing soln prevented development of Coomb's positive red blood cells.	< 1 hr	Up to 7½ mon at -80°C	6-7	Blood collection app, nitrogen freezer, containers Glycerol, 1% fructose and 8% glucose soln, isotonic saline soln, Cr-labeled blood	Transfusions	Valeri (1966)
34. Blood cells, Red	Fresh human blood	Fresh intact red blood cells were washed, suspended in 0.153 M NaCl or 0.138 M NaCl contg 0.017 M EDTA, and stored at 4°C. Hb and hematocrit values and lipid extn were performed on aliquots of the samples, and the remainder of the sample was frozen to -20°C. The frozen samples were analyzed for Hb and lipid content over a period of 16 wk.	Lipid phosphorus and cholesterol were stable for one wk in all experiments. Glycerophosphatide degradation began within two wk, possibly from deacylation. This degradation seems to be catalyzed by Hb.	Loss of phospholipid was not observed with Hb-free red cell ghosts or plasma stored as long as 2 and 6 mon respectively.	>1 hr	2 wk	5	Freezer, plastic syringes, glass containers, refrigerator Na_2EDTA, ACD, NaCl soln with or without EDTA	Biochem study of blood lipids	Ways (1967)

FREEZING (Continued)

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35. Blood cells, Red	Fresh whole human blood	Blood from healthy male donors was collected in ACD-NIH-A anticoagulant or preserved in glycerol with or without slow freezing. After several days of storage, cells were washed either by a continuous wash technique with ionic soln, or by a dilution technique using non-electrolytes with recovery by agglomeration. Washing was carried out under a variety of conditions, and it was evaluated by measurement of intracellular potassium levels and mean corpuscular vol.	No loss of cellular potassium was observed when red blood cells were washed using continuous centrifugation with ionic soln. However, recovery of red cells using agglomeration reduced cellular potassium significantly.	Removal of glycerol from previously frozen red cells should be accomplished by the use of a hypertonic soln.	Few min	5 days-3½ wk	3	Blood collection app, refrigerated centrifuge, refrigerator, Blood Freezing Unit (Int Equip Co), tubes, Cohn blood fractionator, Huggins cytoglomerator ACD-NIH-A anticoagulant; isotonic saline, pH 7.2; 50% aq dextrose soln; 8% glucose and 1% fructose soln; various glycerol soln; sodium-R-lactate soln, pH 7.2; EDTA; $MgCl_2$; KCl	Transfusions	Runck (1968)
36. Blood cells, Red	Freshly drawn human blood	The clinical efficacy of separated red cells, either liq-stored or previously frozen was investigated. Cells frozen by a variety of techniques were included, and clinical findings and practices for use of conc red cells in Vietnam were discussed.	Fresh red cells collected in ACD with part or most of the plasma removed and refrigerated at $4^{o}C$ had a mean storage time of 23 days. Removal of plasma contg isoagglutinins prevented destruction of recipient red cells following transfusion. Frozen cells had an in vitro loss of 26.7% due to processing, and were slow to process. However, frozen cells have been very effective where large amt of blood are needed, and their use cut down on the incidence of hepatitis and blood group isosensitization.	Removal of plasma, which reduced the isoagglutinins anti-A and anti-B, allowed O-negative red cells to be used as universal donor units.	Varied	23 days for liq nonfrozen red cells; not given for frozen red cells		Many methods were explored so equipment varied	Transfusions	Valeri (1968)

FREEZING (Continued)

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37. Blood cells, Red	Freshly drawn human blood	Fresh blood was collected into ACD soln and stored at $4^{o}C$, or it was stored 3 days at $4^{o}C$, then centrifuged. Red cells were mixed with a glycerol-glucose-fructose-Na_2EDTA soln and finally frozen and stored at $-80^{o}C$ for 4 days. Both previously frozen and nonfrozen red cells were washed with a variety of nonelectrolyte soln, and the effect of washing on posttransfusion survival and properties of the red cells was studied.	Washing with all nonelectrolyte soln of both types of blood reduced intracellular potassium levels. Storage for 48 hr at $4^{o}C$ further reduced potassium levels. Loss was greater in previously frozen cells. Washing with 4.5% glucose and 4.5% fructose decreased the mean corpuscular vol and osmotic fragility while increasing mean corpuscular hemoglobin conc and cellular density.	The conc of glucose and fructose in the washing soln was highly critical. 8% glucose and 1% fructose was the best composition for washing red blood cells.	Varied with method	48 hr postthaw storage at 4^{o} C; up to 3 mon frozen	Varied with method	Blood bags, blood collection app, refrigerator, centrifuge, blood freezing unit ACD soln, Na_2EDTA, 50% dextrose, glucose, fructose, isotonic saline, $CaCl_2$, glycerol, albumisol, plasma	Transfusions	Valeri (1969a)
38. Blood cells, Red	Freshly drawn human blood	Human red cells preserved with approx 45% (w/v) glycerol, frozen by the slow freeze-thaw method, and stored at $-80^{o}C$ for up to 6 yr were evaluated for postthaw viability. Two postthaw washing methods, continuous flow centrifugation and agglomeration, were compared, and the effect of postthaw treatment on red cell viability was investigated.	Cells stored up to 6 yr and washed by nonelectrolyte soln and agglomeration did not have as long a posttransfusion survival as those washed by continuous centrifugation. Postthaw stability was 4 hr at $4^{o}C$ in the agglomeration cells and 24 hr at $4^{o}C$ in the centrifuged cells.	Prior dilution of thawed red cells with a 10% glycerol soln was vitally important for maintaining viability during the washing of cells by continuous centrifugation.	2-3 hr	6 hr	Varied with method	Blood collecting app, PR-2 centrifuge, blood freezing unit, refrigerator, Cohn blood fractionator, Huggins Cytoglomerator (Int Equip Co), stainless steel centrifuge bowl Glycerol, glucose, fructose, dextrose, KCL, $MgCl_2$, Sodium-R-lactate, isotonic saline, albumin soln, albumisol	Transfusion	Valeri (1969b)
39. Blood cells, Red	Freshly collected human blood	A plastic bag system made of a thin Kafton-Teflon-FEP double laminate for the deep-freezing of red blood cells preserved by the low glycerol technique was described. The advantages of the plastic container over the previously used aluminum containers were outlined.	The plastic bag was nontoxic, could withstand temp of $+200^{o}$ to $-200^{o}C$, and was easily heat-sealed. Hemolysis was somewhat higher when the plastic bags were used than it was when the aluminum containers were used, but hemolysis levels were still within an acceptable range.	The plastic bags can be used for the agglomerization technique of washing glycerol from red cells.	5-7 min	Samples were thawed after 6 days	Varied	Blood collection app, special plastic storage bags, centrifuge, liq nitrogen freezer, plastic bottles Physiol saline soln, glycerol, fructose, glucose	Transfusions	Akerblom (1970)

FREEZING (Continued)

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40. Blood cells, Red	Whole human blood, some of which is autologous	Several aspects of maintaining blood supplies were presented. The authors felt that more use should be made of storage of autologous blood by patients to be used by them at a later date, especially in diseases like renal insufficiency which can often be predicted in advance.	The authors felt frozen blood still had disadvantages (such as lack of 2,3-DPG after a wk of storage) and that a sufficient supply of liq blood, including autologous blood, should be maintained in a blood bank.			A storage schedule of 1-4 wk was outlined			Maintaining a blood bank for transfusions	Hogman (1970)
41. Blood cells, Red	Freshly collected human blood	Blood collected in ACD was centrifuged, and a soln of glycerol contg glucose and fructose was added to the packed cells. The cells were then frozen to $-85^{\circ}C$. After cells were thawed glycerol was removed from them by dilution with a non-electrolyte soln. Reversible agglomeration after each dilution eliminated centrifugation during glycerol removal. Agglomerated cells were resuspended in physiol saline and were then ready for transfusion.	Blood stored and thawed by this method showed an av 1.45% hemolysis after 2 yr with 87-95% red blood cells present in the recipient's blood 24 hr posttransfusion. 85% of red blood cells was recovered from stored blood *in vitro*. No deterioration was noted in samples stored for 4 yr by this method. 15 units of blood can be thawed and prepared per hr.	Blood stored by this method had a low conc of potassium and virtually no anticoagulant, protein, white blood cells, and blood group isoagglutinins. Blood can easily be shipped, and is free of hepatitis contamination. This method is in use at Mass. General Hospital, Boston.	$< \frac{1}{2}$ hr	Up to 4 yr	4 not counting thawing	Blood collection app, cytoglomerator, disposable plastic blood freezing units, (exact detail of all equipment is given) Physiol saline soln, glycerol, glucose, fructose	Transfusions	Huggins (1970)
42. Blood cells, Red	Freshly collected human blood	500 ml of ACD-collected blood was centrifuged, and 250 ml of the supernatant plasma was removed and stored at $-30^{\circ}C$. An equal vol of a soln of glycerol, sorbitol, and NaCl was added to the remaining cells, and the mixt was frozen in a stainless steel container or aerosol can with liq nitrogen. After thawing, 500 ml bottles or plastic bags were filled with the cell mixt, and the bags were centrifuged and washed twice with sorbitol and physiol saline soln. Then the washed cells were reconstituted with the thawed original plasma.	95-96% recovery of *in vitro* red cells was demonstrated. There was no appreciable difference using either the stainless steel container or the aerosol can, however, the can was much more convenient.	This method is the low glycerol-intermediate cooling method used in Amsterdam.	1-2 hr	Several yr?	6-8	Centrifuge, freezer, bottles, plastic bags, water bath, stainless steel container or aluminum aerosol can Glycerol, sorbitol, physiol saline soln, ACD soln	Transfusions	Krijnen (1970)

FREEZING (Continued)

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43. Blood cells, Red	Freshly collected human blood	An attempt was made to explain the mechanism of freezing injury in red cells by investigating the effect of osmotic stress, salt conc, and thermal shock on red blood cells. The authors rejected the salt conc theory of freezing injury and presented data to support their arguments for freezing injury due to osmotic stress and related phenomena.	Conc of nonpenetrating solutes through freezing reduced total cell water and cell size. When 64% of the cell water was removed, and the size was reduced 45%, resistance to further shrinkage developed leading to an osmotic pressure gradient across the cell membrane. Cells so stressed were subject to thermal shock or to increased permeability to small molecules leading to hypertonicity and hemolysis on thawing.	Cryoprotective agents reduced the amt of ice formed thereby preventing the conc of extracellular solute which in turn prevented the reduction of cell size beyond the tolerable minimum.				Freezer Ammonium chloride, NaCl soln, ammonium acetate, hypotonic sucrose soln	Theory of freeze injury based on osmotic stress	Meryman (1970)
44. Blood cells, Red	Freshly collected human blood	Plastic bags were filled with packed blood cells obtained by centrifugation of ACD-collected blood contg 15-20 vol % of glycerol-sucrose soln. The bags were heat-sealed and cooled between aluminum plates contg liq nitrogen at a rate of $50^{o}C$/min to $-120^{o}C$. Containers were folded and stored in cardboard cartons in a liq nitrogen refrigerator.	The av recovery of red blood cells after freezing, thawing, and washing was 99.25%. No difference was noted between frozen and fresh blood transfused into the same patient. Potassium loss from freezing was 10%, and more was lost during post-thaw storage at $4^{o}C$.		1-2 hr	Not given	5-7	Special freezing app (described), blood collection app, plastic bags, rubber spacers, clamps, cardboard cartons, Latham A. D. Little centrifuge bowl, centrifuge Glycerol, ACD soln, sucrose, NaCl soln, mannitol	Transfusions	Pert (1970)
45. Blood cells, Red	Freshly collected human blood	Extracellular agents such as dextran, PVP, or hydroxyethyl starch, were investigated as substitutes for glycerol in cryoprotection of red cells. The agents were dissolved in ACD mixt into which fresh blood was collected. The blood with cryoprotectant was transferred to an aluminum container which was sealed, coated for insulation, and immersed in liq nitrogen, then stored at liq nitrogen temp.	7.5% of 40,000 mol wt PVP gave a red cell survival of 96.4%, was 84-86% stable in physiol saline soln, and had an in vivo red cell survival rate of 73.5% after 24 hr. After thawing, PVP-preserved blood was stable for 2 wk at $4^{o}C$ but deteriorated badly after that. Hydroxyethyl starch provided 96.7% cell survival, but hemolysis was high. Better results were achieved using packed cells.	The main advantage of extracellular protectants was that they did not make red cells osmotically unstable, and blood could be transfused directly after thawing without washing; also, freezing was very fast.	Few min; 90 sec to freeze	Not given	4-6	Blood collection app, flat metal containers, Linde freezer, liq nitrogen bath, thawing bath Liq nitrogen, methanol, glycerol, PVP, talc or silica, hydroxyethyl starch	Transfusions	Robson (1970)

FREEZING (Continued)

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46. Blood cells, Red	Freshly collected human blood	A special disposable centrifuge bowl was described which allowed centrifugation and gradual glycerolization of up to 42% glycerol. Cells were frozen slowly to -80°C and upon thawing slowly were deglycerolized in the same centrifuge bowl. The effect of this method on metabolism, viability, and postthaw survival time was investigated.	An av of 90.89% red cells were available for recipients after 10 yr storage at -80°C with a mean of 89.23% immediate posttransfusion survival. Red cells were stable in the liq state for several days after thawing if kept refrigerated at 4°C.	Samples stored at 4°C postthaw were recombined with their original plasma to which ACD soln or CPD soln, with or without adenine, had been added. The centrifuge bowl could be used for all major low-temp blood storage methods.	Approx 1 hr	> 10 yr	4-6	Abbott-BRI disposable centrifuge bowls, centrifuge, blood collection app, freezer Glycerol, ACD, CPD, adenine	Transfusions	Tullis (1970)
47. Blood cryoprecipitate	Freshly drawn human blood	An investigation was made of the effect of CPD or acidified platelet-rich plasma on cryoprecipitation of Factor VIII from blood. Cryoprecipitates were prepared in plastic bags or test tubes; the ratio of ACD to blood was 1:6.7, while the ratio of CPD to blood was 1:7.15.	CPD was at least as good if not better than ACD in preservation of Factor VIII. Cryoprecipitation of Factor VIII was adversely affected by low pH. The yield was very low at pH 6.0 and rose to a plateau at pH 6.8 with no further changes until pH 8.0. Acid in acidified plasma may be neutralized with NaOH before cryoprecipitation. Factor VIII was fairly stable at room temp in a variety of diluents.	Both platelets and cryoprecipitate could be extd from the same blood sample without loss of Factor VIII, if a method were developed for neutralization of the plasma in the blood bag after platelet removal.	Not given	24 hr at 37°C	Not clear	ACD soln; CPD soln; normal saline soln; citrated saline; citrated saline, pH 5.9; supernatant plasma; pooled normal plasma	Transfusions	Pool (1967)
48. Blood cryoprecipitates	Freshly drawn human blood	Eight units of whole blood were drawn into bags contg 1 of 4 anticoagulants: ACD, ACD-adenine, CPD, and CPD-adenine. Cryoprecipitates were prepared from each unit of plasma by a modification of Pool's method (1965). Assays for fibrinogen, prothrombin, and factors V, VII, VIII, IX and X were performed on the cryoprecipitates and on the supernatant plasma to see what effect adenine had on the viability of these substances.	Adenine did not alter factor VIII. There was slight to moderate loss of factor V and fibrinogen in the plasma, but prothrombin and factors VII, IX, and X were unaffected. Good factor VIII activity was found in each unit of plasma obtained from double plasmapheresis, therefore both units were satisfactory for use in the prepn of cryoprecipitates.	CPD is as good as ACD for prepn of blood cryoprecipitates. The effect of cryoprotectants and preservatives on blood components other than red cells should be taken into consideration since 'banked' blood is the main source of these components.	1-2 hr	Specimens were rethawed immediately	Varied	Blood and plasma collection app, plastic bags, centrifuge, blood transfer packs, CO_2-acetone bath, ethylene-glycol bath, glass tubes ACD soln, CPD soln, adenine, saline soln	Preservation of blood components other than red cells for transfusions purposes	Graybeal Jr (1969)

FREEZING (Continued)

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49. Blood factors	Outdated ACD-collected blood-bank blood	Coagulation studies were performed upon blood bank plasma removed from settled cells 25-36 days after the blood was originally collected into ACD soln in plastic bags. Assays of clotting factors were also performed on plasma aliquots stored for four months at 4°C and -20°C.	Levels of Factor IX, Factor XI, Factor VII, and prothrombin consistently remained above 70%. Factor V levels were below 30%, and Factor VIII levels averaged 56% with many as high as 70%. No further deterioration occurred after 4 mon at -20°C.		1-2 hr	4 mon	Not clear	Plastic blood bags, centrifuge, refrigerator, freezer, plastic tubes ACD soln	Transfusions for treatment of hemophilia	Rosenthal (1966)
50. Blood factors	Outdated blood-bank blood contg ACD and stored at 4°C	Outdated blood bank blood contg ACD was centrifuged and the plasma separated into a transfer bag. The bags of plasma were submerged in a bath of ethylene glycol at -30°C and completely frozen. At a convenient time the frozen plasma was placed at 0°-4°C for slow thawing. After thawing the plasma was centrifuged, and the Factor VIII-rich precipitate was resuspended in 25 ml of plasma and stored at -30°C until needed for transfusion.	58% of the Factor VIII activity was available after the 21 days of initial blood storage, and 60% of this was recovered by cryoprecipitation. On a vol basis the preserved material had about 4 times the activity of fresh human plasma.	The cryoprecipitate contg Factor VIII (or antihemophilic factor) was used to treat hemophiliacs without causing a circulatory overload.	1-2 hr	Several wk	6-7	Refrigerator, plastic blood bags, transfer packs, centrifuge (PR-2 Int), ethylene glycol bath, deep freeze Supernatant plasma, ethylene glycol	Transfusions for treatment of hemophilia	Weaver (1967)
51. Blood lymphocytes	Freshly collected human blood	A complex method of lymphocyte prepn was described involving centrifugation, agglutination of red blood cells, percolation through a bead column, and further centrifugation and resuspension. DMSO was added to the suspension of lymphocytes and 15 µl samples were sealed in hematocrit tubes; cooled at 1°C/min to -30°C, then more rapidly to -196°C; and stored in liq nitrogen.	Rapidly thawed suspensions which had been maintained at -196°C for 2 min were 85% viable. Frozen cells labeled with chromium-51 gave approx the same assay as unfrozen cells after adjustment for a higher background liberation of isotope in the frozen cells.		4 hr	2 mon	15-16	3 mm glass balls, centrifuge, Pasteur pipette, column of plastic beads in saline buffer, water jacket, incubator, stirrer, hematocrit tubes, heat sealer, ice bath, freezer 3% gelatine in saline, 25% fetal calf serum in tissue culture fluid, IgG fraction of chicken anti-human red blood cell serum, 3% DMSO soln	Histocompatibility typing	Sanderson (1967)

FREEZING (Continued)

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52. Blood plasma	Fresh blood plasma or blood bank plasma	Dried and frozen blood plasma that had been stored from 2-10 yr was evaluated for gross appearance, turbidity, pH, and conc of total protein, albumin, globulin, prothrombin, and complement. Viscosity and electrophoresis were detd on a few samples; and for the dried plasma, residual moisture and solubility were detd.	Dried plasma can be used for transfusions up to 10 yr but loses prothrombin activity after 5 yr. Plasma frozen at -20°C or lower was well preserved after 6 yr and is still useful after 10 yr of storage.		Not given	5-10 yr	Not given	Freezer, lyophilizer, metal or glass containers, rubber stoppers Citrate buffer, ACD buffer	Transfusions	Strumia (1952)
53. Blood plasma	Outdated ACD blood-bank blood	Outdated ACD-preserved bank blood to which additional glucose had been added was centrifuged, and the red blood cells were discarded. The plasma was mixed and divided into 10 ml aliquots. These were stored in screw-capped glass tubes at -10°C. Tubes were removed each day up to 6 mon and analyzed for 15 different protein, enzyme, and nonprotein constituents.	Significant change was noted in nonprotein nitrogen, glucose, and alk phosphatase. Albumin, globulin, total protein, urea, uric acid, creatinine, cholesterol, bilirubin, chloride, amylase, cholinesterase and acid phosphatase showed no significant relative change.	The values given in this paper were comparative initial and final values. Since outdated blood-bank plasma was used, the initial values were not necessarily normal values.	$<$ 1 hr	6 mon	5-6	Centrifuge, pipettes, screw-capped bottles, freezer Glucose, ACD soln	Plasma standards for anal	Walford (1956)
54. Blood plasma	Freshly collected homologous human blood plasma	Fresh-frozen, citrated homologous plasma was used for a priming fluid for clinical cardiopulmonary bypass.	56 patients were perfused with this soln during cardiopulmonary bypass surgery with a fair degree of success.		Approx 1 hr	24-48 hr	Not clear	Semiautomatic pump-oxygenator system, electromagnetic flow meter, water bath, freezer, containers Citrate (ACD?), heparin, calcium chloride	Blood substitute for cardiopulmonary bypass	Davila (1966)
55. Blood plasma	Freshly drawn rat blood	5-10 ml of blood was taken from male adult rats by heparinized syringe. Plasma was immediately separated and divided into 18 portions. Six portions were stored at 22°C, 6 at 4°C, and 6 at -20°C for 1, 2, 4, 8 or 16 days. Electrophoretic studies were performed on samples to determine the effect of storage on plasma proteins.	8-10 proteins were separated, 6 decreased in mobility and the decrease was more frequent in samples stored at -20°C; albumin conc decreased in 2 and 4 days at all temp. Conc of 5 other proteins both decreased and increased as the temp declined. Esterases seemed stable at all temp, but storage time reduced the mobility of 3 isoenzymes.	Different proteins react differently to storage time and/or temp. In general, temp has less affect on plasma proteins than length of storage.	Few min	Samples were held for 16 days	3-4	Heparinized syringe, electrophoresis app, centrifuge, refrigerator, freezer, containers Heparin	Electrophoretic studies of effects of storage and temp on blood plasma	Baker (1967)

FREEZING (Continued)

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56. Blood plasma	Fresh blood from subjects previously injected with heparin	Nine vol of blood from humans who had received parenteral injections of heparin were added to glass tubes contg 1 vol of 0.1 M trisodium citrate. The tubes were stored in ice water, then centrifuged. The resulting plasma samples were stored at -15°C for later anal of heparin-released lipoprotein lipase activity.	Lipoprotein lipase levels in plasma samples frozen for four mon decreased from 10-52% depending on the initial enzyme levels. Av decrease in enzyme activity was about 0.02%/day of storage.		Not given	4 mon	4-5	Blood collection app, glass tubes, freezer, ice water bath, centrifuge 0.1 M trisodium citrate, heparin	Plasma enzyme studies	Boberg (1970)
57. Blood platelets	Freshly drawn human blood	A special app was described for closed-system platelet pheresis which utilizes plastic centrifuge bowls that can be attached to a standard lab centrifuge. The assembled blood bags, centrifuge bowl, and tubes remain connected to the donor throughout, thus allowing reconstituted red cells and plasma to be returned to the right donor easily. The collected platelets can be resuspended for storage in the liq state or frozen in glycerol.	4 whole blood units per donor could be used to extract platelets. 4-unit platelet pheresis from a single donor required about 100 min total time.	This paper described the A. D. Little continuous centrifuge bowl which can be used for washing red cells stored in glycerol as well as for separation of blood fractions.	100 min for collection & centrifugation of 4 units of blood			Special collection and centrifuging app (described), containers Isotonic saline, anticoagulant	Automatic platelet collection for storage	Tullis (1968)
58. Blood serum	Freshly drawn human blood sample	Blood samples drawn for phosphatase anal were allowed to sit at room temp for several hr. The blood samples were also exposed to temp of 0°, 25°, 30°, and 37°C for up to 48 hr, and some serum samples were stored at 4°C under toluene. The effects of these treatments on phosphatase activity were reported.	Only a slight increase in phosphatase activity was noted in sera remaining at room temp up to 6 hr, and 37°C was the preferred incubation temp. Phosphatase activity increased after 6 hr under refrigeration and decreased again after 3-5 days. Toluene did not alter this increase. Thoroughly-separated sera should ideally be kept no more than 1 hr at room temp, or 3-4 hr in the refrigerator. Clinically useful detn of phosphatase may be made on serum kept at 0°C under toluene for 24-48 hr, although results may be 10-20% high.		Few min	Up to 48 hr	2-4	Blood collection app, containers, refrigerator Toluene	Anal of serum phosphatase activity	Bodansky (1933)

FREEZING (Continued)

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59. Blood serum	Nonhemolyzed serum from a cancer patient	Nonhemolyzed serum from a patient with adenocarcinoma of the prostate and skeletal metastases was frozen at -15°C. The effect of frozen storage on acid phosphatase activity was investigated.	Acid phosphatase stability in blood serum was unaffected by freezing and thawing. The enzyme remains stable for at least 112 days when stored at -15°C.		Few min	112 days	2	Blood collection app, containers, freezer	Anal of serum acid phosphatase by the Gutman method (1940)	Davison (1953)
60. Blood serum	Fresh human serum; commercial 30% bovine albumin soln	Methods for preparing and storing clinical standards were the following: 1) Pooled serum free of hemolysis, icterus, or lipemia was collected each day and frozen until 2 liters were accumulated. The pool was thawed, centrifuged, and mixed thoroughly. 2 ml aliquots were stored in corked vials at -10°C without preservative until needed for control serum, 2) Pooled protein-free filtrates were collected after glucose anal were performed and were packaged and stored like the pooled control serum, 3) Standard 6% bovine albumin soln prepared from Armour's 30% soln, was prepared in 25 ml aliquots and refrigerated.	Extensive evaluation of the use of control serum pools for various blood constituents was given.	The main emphasis of this paper was how to establish quality control in a clinical laboratory.	< 1 hr	Serum control pool, 16 mon; albumin soln, not given	Serum control pool, 6-7; albumin, 2	Blood collection app, container, large bottles, freezer, centrifuge, pipettes, corked vials, refrigerator Armour's 30% bovine albumin	Serum anal	Frier (1958)
61. Blood serum	Freshly drawn human blood	Serum was placed in 1.2 ml hard-glass ampules and sealed. The samples were cooled quickly to -196°C.	There was no appreciable change in the conc of glucose, urea, total protein, alk phosphatase, glutamic oxalacetic transaminase, phosphohexose isomerase, or lactic dehydrogenase on fast freezing and thawing. No measurements of qualitative changes were made (protein denaturation, etc).		1-5 min	Several mon	2	Ampules, ampule sealer, Linde liq nitrogen refrigerator Liq nitrogen	Studies of enzyme activity in frozen blood serum	Davies (1965)

FREEZING (Continued)

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62. Blood serum	Whole human blood or serum	This paper described an enzymatic assay specific for pyridoxal phosphate in serum and/or blood. It was found that apophosphorylase b prepn, pH 6.2, stored at $0^{o}C$ lost its activity after 14 days storage; aq pyridoxal 5'-phosphate standard (100 µgm/ml) began deteriorating after 4 wk at $-50^{o}C$. Blood serum specimens contg pyridoxal phosphate and stored at $-50^{o}C$ should be assayed before 3 wk, as they become unstable after this time.	Recovery and assay of pyridoxal phosphate in blood serum specimens stored at $-50^{o}C$ should be carried out within 3 wk after initial storage.	Blood specimens should be light-protected after protein extn.	50 blood specimens/ day can be assayed	Apophosphorylase b, 14 days at $0^{o}C$; pyridoxal-5'-phosphate soln 4 wk at $-50^{o}C$; blood specimens, 3 wk at $-30^{o}C$	4-5 for blood specimens	Freezer, centrifuge, filtration columns, foil, test tubes, hypodermic syringes, reagent bottles, incubator Several, depending on which substance is being prepared	Blood pyridoxal phosphate assay	Hines (1969)
63. Blood serum	Blood-bank serum	Standard secondary sera covering the clinical range of protein values were prepared by a simple method for concentrating serum, based on the fact that solutes are excluded from ice crystals formed when watery soln are cooled to the freezing point. Human blood-bank serum was collected in polyethylene bottles and frozen to $-20^{o}C$. The bottles were placed upside down at $4^{o}C$, and the dripping sera was caught and pooled in a collection vessel.	The degree of conc was dependent on the ambient temp at which freezing and thawing were performed. A more concentrated serum can be obtained by freezing at moderately low temp probably due to a more favorable ice lattice.	This method permits inexpensive prepn of human serum standards.	10 hr		4-5	Freezer, polyethylene bottles	Concentration of human serum	Hollender (1970)
64. Blood serum, Standard soln	Fresh human serum	Glucose and bilirubin were added to pooled human serum to give concentrations of approx 100 mg and 2 mg/100 ml respectively. The bilirubin was first dissolved in a small amt of 0.1 N NaOH, diluted with a little water, then added slowly to the serum with constant stirring. Sodium merthiolate was added to the serum as a preservative. The fortified serum was filtered through a Seitz E. K. filter, and the filtrate was tubed under sterile conditions in 10 ml quantities and stored at $-10^{o}C$.	Blood serum control soln can be shipped and stored for 2-3 mon in advance. Use of a control chart allows a laboratory supervisor to detect changes in the quality of test reagents and to expedite training.	Methods were also given for prepn of serum controls for enzyme anal and protein-bound iodine detn as well as for prepn of a whole blood protein-free filtrate.	½-1 hr	2-3 mon	7-8	Containers, stirrer, Seitz E. K. filter, autoclave, sterile tubes, freezer Glucose bilirubin, 0.1 N NaOH, sodium merthiolate	Standard for blood anal	Benenson (1955)

FREEZING (Continued)

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65. Blood vessels	Whole live rat treated with vasoactive drugs	A vasoactive drug, either serotonin or angiotensin, was injected into rats. This was followed either immediately, or at 5, 10, 15, or 30 min, with an injection of Thorotrast. Within one min after the Thorotrast injection the organ to be studied was removed and frozen in liq nitrogen (or isopentane cooled with liq nitrogen), or it was frozen in situ before removal. After a gross radiograph was taken the organ was sectioned on a cryostat, and microradiography was performed to demonstrate microvascular reactions to the drugs.	This technique permits the observation of both gross angiographic and microvascular reactions in blood vessels as small as 20-30 μ in diam.		Few min (not counting section prepn)	Not given	8-9	Hypodermic syringes; surgical tools; film; cooled saw; cryostat; copper plate, cooled with dry ice, in contact with a max resolution plate covered with celloidin; Siemens AG Cu 3ö x-ray tube Angiotensin, serotonin, Thorotrast soln, liq nitrogen or isopentane cooled in liq nitrogen, dry ice, acetone Gevaert G 209A developer	Study of drug effects on small blood vessels	Kormano (1970)
66. Deoxyribonucleic acid	Purified DNA in standard saline citrate buffer soln, pH 7.0	Calf thymus or phage-T_2 DNA, purified by phenol extn, was exposed to multiple freezing and thawing thus causing a decrease in viscosity. DNA soln was placed in an hermetically-sealed, flat-bottomed retort and frozen to -196°C in liq nitrogen; then it was thawed in a water bath. The process was repeated until the desired viscosity of DNA soln was obtained.	The method allowed degradation of DNA without denaturation, and the bihelical structure of DNA was maintained. However, degradation of DNA did not occur unless the thin layer of frozen material around the flask cracked.	Phenol used for extn may be present as a contaminant.	10-15 min to several hr depending on the no. of freezes and thaws needed to give the proper amt of degradation		3	Liq nitrogen in Dewar flask; nitrogen tank; water bath; hermetically-sealed, flat-bottomed pyrex retorts	Degradation of DNA	Lyscov (1969)
67. Downy mildew	Lima bean seedlings infected with downy mildew colonies	Lima bean (Phaseolus limensis) seedlings were inoculated with zoospores of Phytophthora phaseoli, and colonization was allowed to proceed for 5 days. Seedlings were then placed in a dew cabinet for 24 hr at 20°C to obtain abundant sporulation. Sections of sporulating stems were then put in small bottles and maintained at -13°, -18°, or -60°C.	The organisms were still viable after 476 days when stored at -60°C; 150 days when stored at -13° or -18°C. Percentage of viable sporangia decreased rapidly during the first mon of storage at all temp.	An abundant supply of sporangia can be obtained in 7-10 days by inoculating seedlings with stored sporangia.	6-6½ days	1.3 yr at -60°C	5-6	Lima bean seedlings, dew cabinet, screw-cap bottles, refrigerator, freezer 2% sucrose soln	Epidemiology studies and class demonstration material	Hyre (1968)

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68. Cartilage	Homogenous human costal cartilage	Costal cartilage from a tissue bank was used for the plombage of trepanation defects especially in skull impressions. The cartilage could be used for the plombage of septic cavities by removing necrotic tissue and sterilizing them with antibiotics. Cavities filled with cartilage in this way will gradually heal, and the transplant tissue will calcify.	Cartilage transplants into the bones of patients with chronic osteomyelitis were still intact and calcified after 8 yr.	The method of preserving tissue was not given. We assumed the tissue was preserved by freezing and chem protectants. However, this may not have been the case.	Not given	Not given		Not given	Bone transplantation	Benes (1968)
69. Catalase	Buffered ox liver catalase	0.5 ml samples of M/100-buffered ox liver catalase soln (0.8 μgm/ml) were frozen in test tubes for 10 min by immersion in a bath set to various low temp. Samples were thawed rapidly in a bath at 25°C and, after suitable dilution, the remaining activity was detd in M/75 phosphate buffer, pH 7 at 0°C. Several chem protectants were tested for their effect on the enzyme activity during freezing and thawing.	From -120°C to -192°C catalase solutions retained their activities completely upon freezing and thawing. Catalase became completely resistant to freezing and thawing denaturation after several mon storage in soln at pH 7 or in crystalline states in a refrigerator.	Catalase inactivation upon freezing was dependent upon temp, conc, and pH. Concentrated enzyme was more resistant to freezing.	Varied	Several mon	3-4	Test tubes, constant temp bath Catalase, phosphate buffer, 1 M glycerol, 1 M glucose, 0.1 M sodium acetate, 0.1 M ammonium sulfate, 0.1 M sodium butyrate, 0.1 M sodium caprylate, 5×10^{-4} M sodium dodecyl phosphate	Maintenance of enzyme activity in preserved tissue samples	Shikama (1961)
70. Cell nuclei	Isolated cell nuclei	Two procedures were used for isolation of nuclei, both involving purification by sedimentation through conc sucrose soln. One method used white saponin for cell lysis, while the second used mechanical disruption of cells in a homogenizer. Liberated nuclei were centrifuged, washed, and suspended in a mixt of 95% glycerol contg 5 mM $MgCl_2$ and potassium phosphate buffer. The suspension was stored at -20°C or -196°C.	Saponin was a useful lytic agent for isolation of cell nuclei. Its use did not affect the RNA polymerase activity of rat liver nuclei and other nuclei. Enzyme activity was preserved in cell nuclei stored for 8 wk in 70% glycerol soln contg a divalent metal and phosphate buffer at low temp.		Few min (not counting isolation of nuclei)	8 wk	2-3 (storage only; not sepn)	Centrifuge, Dounce homogenizer, cold bath, tissue press, centrifuge tubes, pestle, cheesecloth filters, ampules or containers Culture media, sucrose soln, white saponin, EDTA, potassium phosphate buffer, 95% glycerol, $MgCl_2$	Studies on RNA polymerase	Read (1970)

FREEZING (Continued)

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71. Cell suspensions	Cell suspensions of blood and yeast; water droplets	A centrifugal method (described) for ultrarapid freezing of cells and subcellular particles was developed in which small droplets were centrifuged at high speeds through liq nitrogen. Material such as blood and water were injected from a syringe or sprayer into a rotor. As the rotor speed increased smaller frozen droplets were formed.	Cell survival was inversely related to increasing droplet size, and directly related to rotor speed and warming rate. Survival rate of yeast cells was 33.8%.		½ hr	Not given	3	Special centrifuge in liq nitrogen (described), containers, hypodermic syringe, sprayer, air filter Triply distd water	Studying effects of ultrarapid freezing on cells; also maintenance of cell lines for future use	Anderson (1966)
72. Cerebrosidase (β-galactosidase)	Fresh rat brains	Cerebroside β-galactosidase was isolated from rat brain. Electrophoresis yielded a major and a minor fraction. The major fraction was separated on a DEAE-Sephadex column to yield 3 fractions. The major fraction from this column can be purified further by precipitation of impurities at pH 5. The crude enzyme was fairly stable to storage at 4°C, but exposure to ion-exchange made it unstable. 50% glycerol preserved the cerebrosidase fairly well at -20°C without the mixt freezing.	The enzyme purified by ion-exchange was unstable to storage at -20°C, but the addition of 50% glycerol and 1% galactose kept it stable for several mon.		Few min (not counting enzyme isolation)	Several mon	2	Freezer 50% glycerol, 1% galactose soln	Study of action of β-galactosidase on cerebrosides	Bowen (1968)
73. Chloroplasts	Chloroplast suspension	Spinach chloroplasts were prepared in a medium of 350 mM sodium chloride and 40 mM Tris-hydrochloric acid buffer, pH 7.5. Nine vol of a soln of 50% glycerol (v/v), 125 mM potassium chloride, and 20 mM Tris-hydrochloric acid buffer, pH 7.5, were added to the chloroplast suspension, and the mixt was stored at -20°C.	There was a 50% loss of photophosphorylation during the first wk of storage, and 25% additional loss after 2 wk. Photosynthetic electron flow and light-induced conformational changes were lost more slowly than ATP hydrolysis or synthesis in stored, glycerinated chloroplasts.	Light-induced conformational changes were a more sensitive test for assessing the high energy state of spinach chloroplasts than reactions involving ATP hydrolysis or synthesis.		Up to 6 mon	3-4	Shaker, flasks, freezer Sodium chloride, 50% glycerol soln, potassium chloride, Tris-hydrochloric acid buffer	Study of photophosphorylation in chloroplasts	Packer (1966)

FREEZING (Continued)

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74. Coccidial sporozoites	Eimeria meleagrimitis and E. tenella cells suspended in media	Coccidial sporozoites, and sporocysts contg sporozoites, were suspended in media contg 7% DMSO and frozen to -80°C at 1°C/min; the samples were then stored in a cylindrical tank above liq nitrogen.	After 1 mon there seemed to be no significant difference between frozen and nonfrozen sporozoites in patterns of development and infection of bird hosts. Frozen sporocysts fed to birds produced oocysts which in turn caused a less intense infection than nonfrozen sporocysts.	This method permits easy maintenance of stock cultures and makes it possible to work with parent strains for an extended time.	2-2.5 hr	Longer than 1 mon (probably much longer)	4	Liq nitrogen freezer with thermostat for lowering temp gradually, cylindrical storage tank DMSO, liq nitrogen	Maintenance of stock cultures	Doran (1968)
75. Coccidial sporozoites	Sporozoite suspension	Excysted sporozoites of Eimeria adenoeides, E. mivati and E. tenella were suspended in media contg 2.5, 5, 7.5, 10, 12.5 and 15% DMSO in screw-cap tubes. The tubes were frozen to -30°C at 1°C/min, then 10°C/min down to -80°C. The tubes were then stored in liq nitrogen vapor.	At room temp, low conc of DMSO had the best effect on sporozoite survival. Frozen sporozoites were protected best by high conc of DMSO. The rate of cooling from 0° to -30°C was important. More than 70% of each species survived thawing under the most favorable conditions.		1-2 hr	> 4 days	5-6	Grinder, centrifuge, screw-top tubes, biol freezer Ringer's soln, trypsin blue soln, DMSO, culture media, liq nitrogen vapor	Maintenance of viable sporozoites	Doran (1969)
76. Corneas	Whole human eye-bank corneas with small limbal rims	Corneas from 4 eye-bank eyes were excised with a small limbal rim. Time between death and enucleation of the specimen varied from 4-12 hr. Some corneas were put on ice 5 min then frozen and stored at -20°C in a cold moist chamber; others were lyophilized and stored at room temp or dehydrated with a mol sieve and 95% glycerine. Respiratory enzymes were measured as an indicator of viability of samples stored by each method.	Storage up to 3 days in a cold, moist chamber, or up to 5 mon by lyophilization, decreased the amount of respiratory enzymes only moderately. Whereas storage with the mol sieve and sterile glycerine led to complete loss of enzymatic activity in the corneal stroma thus indicating that corneas stored in glycerine with a mol sieve are not viable transplants, but act only as a scaffold.		Not given	From 3 days to 5 mon depending on method	Up to 6	Surgical tools; cryostat; Dewar flask or container; moist, constant temp storage chamber; liq nitrogen freezer; air-tight containers; mol sieve; lyophilizer Dry ice, liq nitrogen, physiol saline soln, 95% sterile glycerine	**Corneal Transplant**	Blodi (1968)

FREEZING (Continued)

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77. Endocrine glands	Fresh ovarian and testicular tissue from rats	Ovaries and testes from 1 wk old rats were cut in two, soaked in 15% glycerol-saline, and were gradually frozen to -79°C and kept for 1 hr. The testis tissue was also frozen to -190°C for varying periods. Later the tissues were thawed and placed under the skin of ovariectomized female rats and castrated male rats respectively to check for viability.	Although many follicles were destroyed during freezing and thawing, those that survived continued to grow in the ovariectomized rats. Testis tissue grafts readily 'took' after being frozen from 1 hr up to 7 wk.	The preservation method appeared to be more useful for testis tissue than it was for ovarian tissue.	Not given	1 hr-7 wk or more	3	Surgical tools, dry ice or liq air freezer with temp control, containers 15% glycerol-saline soln	Tissue grafts	Deanesly (1954)
78. Endocrine glands	Fresh ovarian autograft; testicular tissue from infantile male rats	Rat ovarian tissue and testicular tissue were slowly frozen in liq air to -190°C in 15% glycerol-saline soln. Implants of the stored ovarian tissue caused oestrous cornification in ovariectomized animals in 6-24 days. Implants of the testicular tissue into castrated adult males readily formed homografts.	Rat ovarian tissue stored up to 1 yr still caused reappearance of oestrous cornification in ovariectomized animals.	Other endocrine and related tissues can probably be preserved in a like manner for use in grafting.	Not given	> 1 yr; indefinitely	3	Special cooling vessel (described) or nitrogen freezer, surgical tools, containers 15% glycerol-saline soln, liq air	Transplantation of sex glands	Smith (1954a)
79. Endocrine glands	Freshly excised rat ovaries and infantile testicular tissue	Ovaries removed from rats were treated with glycerol, frozen, and stored at -79°C or -190°C for periods varying from 1 hr to 1 yr. The ovaries were then thawed and reimplanted subcutaneously. In some cases the ovarian tissue was homografted and in others autografted into the animal from which it was taken. Some homografts were implanted into the same strain and some into other strains. Infantile testicular tissue treated with glycerol and transplanted into adult animals was also investigated.	Viability of frozen ovarian tissue was maintained even after storage in 15% glycerol at 190°C for 1 yr. Daily vaginal smears studied for months after implantation of the frozen tissue showed that oestrus cycles recurred regularly in many of the recipients. All stages of spermatogenesis followed testicular tissue transplantation into the scrotum of castrated rats.	Several other endocrine gland tissues were frozen in glycerol with varying results upon reimplantation.	1-2 hr	Up to 1 yr	3-4	Surgical tools, freezer Glycerol	Study of cell survival at low temp	Smith (1954)

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80. Enzymes, Proteolytic	Trypsin-substrate soln at pH 7.5	The transfer reaction between amino acid ester and hydroxylamine which is catalyzed by trypsin was investigated for its reaction to cold. Three trypsin substrates, lysine ethyl ester, lysine methyl ester, and arginine methyl ester, reacted more rapidly with hydroxylamine in the presence of trypsin at -23°C then they did at 1°C. Benzoyl L-arginine ethyl ester formed more hydroxamic acid in the presence of trypsin at 1°C initially than at -18°C, however, hydrolysis to the amino acid occurred. This change did not occur in the frozen state. This shows that freezing can accelerate catalyzed reactions.	Rates of hydroxylaminolysis of trypsin were roughly twice that of the same compds at 1°C. Relative rates of the three substrates differed also in the two systems.	Enzyme reactions may speed up or change their pathway in the frozen state.	Few sec	Not given	3	Tubes, acetone-dry ice bath, constant-temp chamber, water bath Lysine ethyl ester, lysine methyl ester, arginine methyl ester, benzoyl-L-arginine ethyl ester, hydroxylamine, phosphate buffer, trypsin	Study of enzyme reactions in cold	Grant (1966)
81. Enzymes, Yeast	Yeast paste	Yeast paste prepared from Candida pseudotropicalis was suspended at room temp in sodium phosphate, pH 7.0. The paste was brought to 34°C in a water bath, and toluene was added. The mixt was stirred, then diluted with sodium phosphate to pH 7.0 at 4°C. Beta-mercaptoethanol was added, and the mixt was stirred overnight, then centrifuged. The supernatant gave a crude ext. This was further purified with $(NH_4)_2SO_4$, EDTA, and column chromatography, and finally the resulting soln contg uridine diphosphate galactose 4-epimerase was stored at -90°C.	A 31% enzyme yield with specific activity ranging from 41.4-87.4 protein units/mg was obtained.			24 hr at 4°C	>10	Water bath, stirrer, centrifuge, ice bath, tissue homogenizer, DEAE-cellulose column Phosphate buffer, β-mercaptoethanol, $(NH_4)_2SO_4$, succinate buffer, EDTA, NaCl, HCl	Yeast enzyme studies	Darrow (1968)
82. Escherichia coli	E. coli suspension in culture medium	Drops of a culture were allowed to fall from a pipette (36 drops/ml) into a Dewar flask containing liq nitrogen. 300 or more drops were transferred to a container previously cooled to -70°C and maintained at this temp until used.	E. coli B maintained 30% viability after 2 yr storage at -70°C. E. coli K12 HfrC maintained 80% viability after 1 mon storage at -70°C.	This method allows source material from a single culture to be stored unchanged.	1-2 min	2 yr	4	Dewar flask with liq nitrogen, freezer, storage flasks or bottles, pipettes	Maintenance of cultures	Cox (1968)

FREEZING (Continued)

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83. *Escherichia coli*	Cells suspended in 2% gelatin	*Escherichia coli* ML30 cells were suspended in 2% gelatin and freeze-dried for 8 hr at constant platen temp of 49°C and pressure of 10 µm of mercury. The moisture content was less than 1.5%. Cells were also frozen at -40°C, and non-dried or frozen cells were used for controls. Effects of freeze-drying on enzyme induction, membrane permeability, RNA synthesis, protein synthesis, and susceptibility to antibiotics were investigated.	0.6% cells survived and 23% of the survivors were metabolically damaged after lyophilization for 8 hr at 49°C. Permeability was altered, RNA leakage occurred, cells were susceptible to antibiotics, and ribosomal degradation occurred. Cells frozen (only) at -40°C in 2% gelatin showed no appreciable metabolic damage or death.	RNA damage had to be repaired by the cells before protein synthesis and growth could resume. Nitrogen sources stepped up this process. Glycerol and glucose were better carbon sources than sodium lactate or sodium succinate for freeze-drying cells.	8 hr	Not given	4-5	VirTis freeze dryer, centrifuge, drying oven 2% gelatin, MB (MM without agar), TSY (Trypticase soy agar with 0.5% yeast ext), MM (sodium citrate, 2.5; ammonium sulfate, 1.0; potassium phosphate, 2.0; sodium chloride, 1.0; glycerol, 2.0; and agar, 15.0 gm/l)	Study of freezing and lyophilization effects on bacteria	Sinskey (1970)
84. Eye lenses	Fresh or refrigerated enucleated human eyes	Normal human lenses were obtained immediately after enucleation of eyes or after storage of the eyes 12-18 hr at 4°C. After weighing, lenses were stored at -25°C under nitrogen until used. Chloroform ext of lipids from the lenses were also stored at -25°C.	Phospholipid composition was not altered during storage of eye lenses at -25°C.		Few min	Not given	3	Surgical tools, refrigerator, liq nitrogen bath Liq nitrogen	Study of ageing of lens tissue	Broekhuyse (1969)
85. Fish	Fresh caught *Trematomus* fish	Antarctic fish of the *Trematomus* species were caught by line or trap. Blood was immediately collected by cardiac puncture and allowed to clot at 0°C for 6 hr. Serum freezing points were measured, serum calcium was detd, and protein and carbohydrates were analyzed.	Serum freezing points of these Antarctic fish indicated that they do not freeze at -1.87°C because their blood is isosmotic to sea water. A glycoprotein in the serum accounted for 30% of the freezing point depression of the serum.	These fish frequently live on anchor ice but do not freeze.		Can survive at -1.87°C without freezing		Fishing gear, wire fish trap, hypodermic syringes, Fiske osmometer, refrigerator 10% trichloracetic acid	Study of freezing resistance in Antarctic fishes	DeVries (1969)
86. Fish	Whole live killifish	Physiochem properties (serum osmolality and blood pH), serum inorg ions, and tissue water were studied in parallel groups of adult male killifish acclimated to temp ranging from 30° to -1.5°C in salt water under otherwise constant lab conditions.	When the temp surrounding the fish was lowered from 20° to -1.5°C, serum osmolality increased by 20% and inorg electrolytes dropped from 98 to 93%. Serum electrolytes increased except for potassium and inorg phosphate levels which were unchanged. Water content decreased 8% and blood pH was higher at 10°C than at any other temp.	Osmotic and ionic regulation were not as effective in the cold, but they were not poor enough to cause death by osmotic imbalance.		Fish were kept at various temp from 1 day to 51 wk	2-3	Salt water aquarium with temp control, gallon jar Aronson's feed mixt, frozen brine shrimps	Study of cold adaptation in killifish	Umminger (1969)

FREEZING (Continued)

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87. Flexibacteria	Cell suspensions in culture media	28 species (91 strains) of flexibacteria, grown on special media and pipetted or centrifuged off, were frozen in liq nitrogen (-196°C) and most survived without protective additives. 10% glycerol or 10% DMSO permitted survival of some of the sensitive strains but adversely affected some others. Storage of organisms at -22°C was not as satisfactory, as most strains died after a wk or two. Others lasted for at least 21 wk.	Most of the organisms frozen at -196°C produced living cultures after 1 yr storage. Organisms stored at -22°C died within 1-21 wk.	Dense suspensions of cells froze better than dilute ones. Cryoprotective agents must be tested on each species and strain for protective effectiveness.	Few min	1 yr or longer	3-4	Pyrex tubes, cotton plugs, centrifuge, pipettes, incubator, liq nitrogen freezer, standard freezer, water bath, sterilizer Special nutria media, 10% DMSO soln, 10% glycerol soln, liq nitrogen	Species or strain maintenance	Sanfilippo (1970)
88. Foods	Varies with type of food being frozen	The food or product to be frozen entered the warm end of a tunnel on a belt. Gaseous cooling was continued until half the heat was removed from the specimen. Then fine droplets of liq nitrogen were sprayed on the specimen causing rapid boiling and generation of nitrogen gas until the product was frozen. The thermodynamics and processing were discussed.	The freezing tunnel operated at 85% efficiency in heat exchange. Samples lost up to 12% of their wt depending upon the type of food or sample. Samples frozen too rapidly crazed, ruptured, or curled.	Most foods stood up better if the temp of both specimen and gas were lowered gradually. 1% nitrogen gas was lost purposely to prevent infiltration of ambient air into the tunnel.	Few min	Not given	2	Freezing tunnel equipped with conveyor belt and liq nitrogen spray (described) Liq nitrogen	Food preparation	Alkire (1968)
89. Fruit, Tomato	Whole immature fruit	Wedges of tomato parenchyma from immature fruit, or the whole fruits themselves, were subjected to various freezing rates and thawing conditions. Freezing rates ranged from <0.1°C/min to >100°C/min. The effects of freezing and thawing on protoplasm and ice crystal patterns were observed.	A slow freezing rate resulted in enlarged intercellular spaces and sepn or breakage of cells, while a fast freezing rate did not. Rapid freezing resulted in very small ice crystal formation, and rapid freeze-moderate thaw caused the least damage to cells and the least disorganization of protoplasm.		Few min-2 hr	Tissue was thawed after 1 hr	2-3	Copper-constantan thermocouples, Rikadenke recording potentiometer, liq nitrogen container, freezer Liq nitrogen	Study of freeze-thaw regimes on plant cell parenchyma	Mohr (1969)

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90. Fungi	Agar culture mycelial plugs	Agar-culture mycelial plugs were cut from fungal cultures with a cork borer. 4 such plugs were transferred to glass ampules which had previously been labeled, plugged with cotton, filled with 0.8 ml 10% glycerol soln, and autoclaved. Ampules were heat sealed and cooled at $1^{o}C$/min to $-35^{o}C$; then they were frozen to $-196^{o}C$.	Specimens stored 18 mon produced normal cultures when cultivated immediately after thawing. However 2/3 of the cultures showed a decrease in radiating growth as compared to controls.		$1\frac{1}{2}$-2 hr	18 mon	4-5	Ampules; culture dishes; cork borer, #3; autoclave; pipettes; heat sealer; Linde BF-2 freezer; Linde LNR 250 liq nitrogen freezer 10% (v/v) glycerol soln, various culture media	Maintenance of type cultures	Hwang (1968)
91. Gastric secretions	Gastric secretion from dog	Gastric secretions were collected from a cannula inserted into either a canine gastric pouch or fistula. The samples were adjusted to pH 1.8 with acid and were either left at room temp or were cooled to 4^{o}, 0^{o}, -4^{o}, or $-20^{o}C$ with albumin and/or acidified glycerol added as cryoprotectants. Pepsin activity was measured in samples stored at the various temperatures.	Canine gastric samples containing pepsin can be stored 3 hr at $0^{o}C$ without preservatives, or up to 1 day at $-20^{o}C$ in the presence of acidified glycerol with almost no loss of enzyme activity. 75% enzyme activity remains after 20 days at $-20^{o}C$ in the presence of acidified glycerol.	Albumin was not necessary as a preservative because sufficient protein was available in the sample to offer protection. Species differences in storage of gastric soln have been reported.	$\frac{1}{2}$ hr	3 hr at $0^{o}C$, 1 day at $-20^{o}C$, 20 days at $-20^{o}C$ with 75% enzyme activity	2	Surgical tools, ice bath or freezer, storage containers HCl, glycerol, albumin	Anal of pepsin activity	Hunner (1969)
92. Grass and clover herbage	Fresh mown grass and clover	Herbage for ruminant nutrition studies was cut, thoroughly mixed, divided into 100-150 gm fresh-wt samples, and sealed in polythene bags. The bags were placed in a freezer at $-20^{o}C$ after fresh wt anal had been performed on one of the samples. After 24 hr in the freezer the samples were thawed and left in the atmosphere at room temp for varying times, after which analyses were made to see what changes, if any, occurred.	Invertase activity was noted in both grass and clover after thawing. By 24 hr postthaw there was considerable loss of carbohydrate in grass but not in clover. Total nitrogen remained constant up to 60 hr postthaw, but extractable nitrogen was reduced upon freezing. Freeze-stored herbage samples should be consumed within 24 hr postthaw.	This study investigated the conditions necessary to insure that thawed herbage did not change its postthaw nutritional composition before it was consumed.	$\frac{1}{2}$ hr	24 hr postthaw	5-6	Grass cutter, polythene bags, freezer	Animal feed studies	MacRae (1970)

FREEZING (Continued)

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93. Guinea worms	D. medinensis embryos in distd water	Dranunculus medinensis embryos in sterile distd water were placed in ampules contg about 2000 embryos/ml and cooled to -78°C at 5°C/min. No additives were used.	A 1% infection rate was observed in cyclops infected with embryos stored for six months; one larva was released from a cyclops infected with embryos stored for 40 mon. This is much lower than the infection rate obtained with cyclops infected with fresh embryos.	A similar technique has been used for Wuchereria bancrofti, Dirofilaria immitis, Brugia pahangi. Although the infection rate was low, the technique is still effective for laboratory maintenance and study of the organism. The technique may be applicable to other parasitic nematodes with free-living larvae.	15-30 min	6 mon-3 1/3 yr		Freezer or liq nitrogen, ampules Sterile distd water	Maintenance of strain	Muller (1970)
94. Heart	Freshly excised frog heart	Frog heart was removed, placed in Ringer's soln, and trimmed. The sinus venosus, with about 2 mm of the upper venae cavae attached, was dissected and transferred to fresh Ringer's soln where it continued beating actively. The ventricle was placed in Ringer's soln and began beating after stimulation. All pieces (in several sizes) were placed in 30-100% ethylene glycol for less than 1 min to 10 min, then blotted to remove excess fluid. The pieces were frozen in liq nitrogen, then thawed and observed for resumption of activity.	65% of the sinus venosus pieces and 57% of the ventricular pieces resumed beating after freezing. Pieces beat from 1-2 days after thawing. All conc of ethylene glycol were effective if pieces were exposed more than 1 min. 5 min exposure for sinus venosus and 10 min for the ventricle were optimum.	The cryoprotectant provided only surface protection. A method is needed to supply protection throughout.	10-20 min	Heart pieces beat 1-2 days after freezing and immediate thawing	5-7	Surgical tools, Petri dishes, liq nitrogen container, wire loop Ringer's soln; ethylene glycol, 30, 60, and 100% soln	Study of freezing effects on heart beat	Luyet (1969)

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FREEZING (Continued)

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95. Heart	Freshly excised frog hearts	Frog hearts were excised and placed in frog Ringer's soln. The hearts were transferred at 10 min intervals into gradually increasing conc of glycerol or ethylene glycol cryoprotectant at room temp. They were then cooled to $0^{o}C$ and transferred again into gradually increasing conc of cryoprotectant. The temp was lowered to $-55^{o}C$ at $5^{o}C$/min intervals. Some hearts were then immersed in liq nitrogen. Thawed tissues were examined microscopically for damage.	90% of the sinus venosae and 35% of the atria beat spontaneously 1-2 hr after rewarming. None of the ventricles recovered their beats even with stimulation. Much ice damage was observed in the heart tissues that had been frozen.		Several hr	Not given	7-10	Cooling bath, liq nitrogen freezer Frog Ringer's soln, glycerol, ethylene glycol	Study of freezing effects on heart tissue	Rapatz (1970)
96. Heart valves	Freshly excised human aortic valves	Homograft aortic valves were immediately frozen to $-70^{o}C$, then carefully packed in 2 polyethylene containers made from flattened tubes in such a way that an air cushion surrounded the tissue and protected it from damage. The whole package was stored in a freezer until it was sterilized by 2.0 megarads of high level radiation and used.	Not given	Deep-frozen aortic valve homografts are very brittle and fragile and are often damaged in handling. This method of packaging prevents this.	10 min	Not given	4	Nitrogen freezer, surgical tools, polyethylene tubing, heat sealer, electron beam energy source, glass indicator beads	Heart valve transplants	Cortes (1969)
97. Heart valves	Freshly excised bovine heart valves	Several commonly used methods of tissue preservation including chemical sterilization with β-propiolactone, freezing, freeze-drying and combinations of these were investigated to determine the best method for preserving the biol and phys integrity of beef homograft cardiac valves.	The authors concluded that none of the methods tested were optimal. Tensile strength and elasticity were reduced to some extent by all the combinations tried.	A simplified method for testing tensile properties of biol tissue was described.	Varied	Not given	Varied	Varied with method β-propiolactone, (others not clear)	Heart valve transplants	Firor (1970)

FREEZING (Continued)

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98. HeLa cells	HeLa cell suspension in liq medium	Tang trachoma agent which had undergone six or more serial passages in HeLa cells was used. The infected cells were suspended in Hank's balanced salt soln without bicarbonate, with 0.5% (w/v) lactalbumin hydrolysate and 25% horse serum. DMSO was added, and specimens were cooled to -50°C at the rate of 1°C/min, then stored in ampules in dry ice or liq nitrogen. Infectivity titres were measured as an indication of viability.	61% infectivity remained in samples frozen slowly to -196°C and stored for 42 wk. Slow loss of infectivity occurred in samples stored at -79°C over dry ice.	Freezing of inclusion-bearing cells, rather than freed particles, was more effective as the cell medium seems to offer protection against damage.	1-2 hr	Up to 1 yr	4-5	Ampules, cooling bath (Nagington and Greaves), freezer Hank's modified balanced salt soln, lactalbumin hydrolysate, horse serum, DMSO, liq nitrogen, solid carbon dioxide	Maintenance of cell line for research	Reed (1966)
99. Kidney	Freshly excised canine kidney	Left kidneys were removed from mongrel dogs and perfused with standard dextran soln contg 15% DMSO at 20°C for 15 min. The kidney was placed in a sterile rubber glove and frozen at -10°C or -50°C. After thawing, the kidney was autotransplanted. A 25% soln of mannitol was given intravenously just before vascularization, and the effect of this on renal and ureteral swelling was observed.	After this treatment renal swelling seemed less severe and ureteral swelling was definitely decreased. Uniform ureteral survival and preservation of numerous tubules and glomeruli were noted, but transplanted kidneys did not function.		1 hr	Storage time was up to 144 hr; kidneys did not function however	5-6	Surgical tools, sterile rubber glove, freezer Dextran soln contg 15% DMSO, 25% mannitol	Transplants without osmotic swelling	Barner (1965)
100. Kidneys	Cannulated whole rat kidney	The renal artery of an anesthetized rat was cannulated, then the kidney was removed. The cannula was connected to a perfusion app, and the kidney was suspended in a cooling jacket. The kidney was perfused with a soln contg gradually increasing conc of DMSO as it was cooled to -25°C then was frozen to -79°C. Upon rewarming perfusion was recommenced, and DMSO was removed from the kidney. This technique kept wt gain and protein loss in the kidney to a minimum.	Kidneys were kept for 30-60 min in the frozen state.	Kidneys were not reimplanted to test their viability. However, wt gain, histological changes, and protein loss were measured or observed.	$< \frac{1}{2}$ hr	At least 1 hr	4-5	Surgical tools, cooling jacket, perfusion app (Palmer timed injection app) Dry ice, methylated spirits, mannitol, DMSO, perfusion fluid (described)	Kidney transplants	Carruthers (1969a)

FREEZING (Continued)

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101. Kidney	Whole rat kidney and ureter	Rat kidney was cannulated through the renal artery and removed along with the catheterized ureter. The cannula was connected to a perfusion app and perfused with solutions contg dextran (mol wt 110,000) and increasing amounts of DMSO in 5% increments up to 40%. 2 ml of soln were perfused at each step. As DMSO conc was increased, temp was gradually lowered to -25°C. At this temp perfusion was stopped, and the kidney was frozen to -79°C. After 1 hr the kidney was thawed, rewarmed, and the DMSO removed. The kidney was then linked by the cannula to the aorta of a 2nd rat, and the excreted urine was collected.	13 kidneys were maintained 1 hr at -79°C; 5 showed preservation of tubular function while 8 did not.		Not given; $<$ 1 hr	1 hr	5-6	Perfusion app, surgical tools, cannula, catheter, thermos flask with dry ice, cooling bath, Millipore filter Perfusion fluid contg dextran and 5-40% DMSO with or without 5% rat serum, dry ice, heparin, oxygen	Kidney transplants	Carruthers (1969)
102. Kidney, heart, and liver	Solid blocks of rabbit tissue	Blocks of rabbit kidney, heart, and liver were either powd; coated with talc, flour, or starch; or coated with Cellusolve (ethylene glycol monomethyl ether) and immersed in liq nitrogen. The coated samples were compared to samples cooled in isopentane or liq nitrogen alone by measuring the cooling rate with a copper constantan thermocouple inserted into the center of each sample.	The powd-coated samples cooled twice as fast as the hydrocarbon-bath samples and 4-5 times as fast as Cellusolve-coated samples; the morphology of all samples was about the same. Talcum powd was the best coating agent.	This technique was recommended as a substitute for hydrocarbon baths cooled in liq nitrogen when safety, simplicity, and speed are needed. It is important to pass through the temp range of -30°C to -40°C as this is the range where ice crystals increase rapidly.	Few min	Not given	2	Dewar flask of liq nitrogen, container for coating material, forceps, thermocouple Liq nitrogen, talc, flour, sugar, starch, other coatings, Cellusolve, isopentane	Tissue fixation for microscopy	Moline (1964)
103. Kidney, spleen, or ileum	Whole human spleen or kidney; ileum segments	Dog kidneys, spleens, and ileum segments were preserved with DMSO soln with or without dextran. Then they were frozen at 1°C/min. The frozen organs were later thawed with a diathermy machine.	The organs testes survived an av of 36 hr, but most were damaged and would not function on transplantation. Thawing by use of a diathermy machine was an unsatisfactory method.	The authors concluded that whole organ bank techniques could not exist with the knowledge now available (1964); and that the fundamentals of freezing and thawing effects on tissue must be further elucidated.		36 hr av	3	Freezer, diathermy unit 6 or 12% DMSO, 10% dextran	Whole organ bank	Manax (1964)

FREEZING (Continued)

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104. Insects, Ants, wasps, and bees	Whole live insects	Carpenter ants, ichneumon wasps, and honey bees were cold-stressed by placing them in precooled glass vials contg wood shavings. Vials were cooled from $4^{o}C$ to $-3^{o}C$ in an ice bath and stored in an ice chest in a cold room. Cold-stressed insects were weighed and extd in a homogenizer with methanol-water soln. The resulting suspension was centrifuged to remove debris, deionized, and prepared for anal of its glycerol triacetate content by gas chromatography. Other metabolites were also measured.	Glycerol accumulation commences immediately upon cold stress; the buildup induced by temp from $4^{o}C$ to $-3^{o}C$ was quite uniform and the polyol was equally distributed throughout the insects' bodies. Glycogen levels decrease as glycerol levels increase, and glycogen may be the precursor to glycerol. Bee and ant glyceraldehvde-3-phosphate dehydrogenase was kinetically different from yeast and mammalian forms of the enzyme, and it maintained its activity below $0^{o}C$. Hibernation character and glycerol accumulation were related in ichneumon wasps and suggest that glycerol is important in the overwintering process.		Not clear	Ants were stored up to 1 yr at $4^{o}C$	Varied	Glass vials, wood shavings, ice bath, ice chest , cold room, thermometer Ammonium sulfate	Study of glycerol metabolism in insects during cold stress	Nordin (1970)
105. Intestines	Either biopsy sample, or specimen from subject that had died 2 hr previously	A biopsy of distal duodenal or proximal jejunal mucosa was performed by means of a Crosby pre-oral biopsy capsule. The tissue was rinsed in 0.85% saline, blotted to remove excess mucous, frozen on dry ice and stored at $-20^{o}C$ until assayed for disaccharidase activity. Sections of necropsy whole-thickness bowel wall were also frozen at $-20^{o}C$.	Storage at $-20^{o}C$ for up to 2 wk did not affect disaccharidase activity in biopsy material; necropsy material gave consistent analyses up to 6 wk.		Few min	2 wk	3	Crosby pre-oral biopsy capsule, freezer, surgical tools, containers Dry ice, 0.85% saline soln	Assay of disaccharidase activity including lactase, sucrase, palatinase and maltase	Arthur (1966)

FREEZING (Continued)

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106. Intestines	Fresh sections of large and small human intestines	Samples of the proximal jejunum, terminal ileum, and colon of newborn, 4-, 7-, 10-, 14-, and 28-day rats, as well as additional samples from 21-day fetuses, adult, and germ-free adult rats, were immediately frozen in a dry ice-acetone bath and stored at -70°C. Frozen sections were cut in a cryostat at -15°C, incubated on various substrates for 4.5 hr at either 37° or 23°C, counterstained with hematoxylin and eosin, dehydrated with alc, and cleared in xylol. Intestinal glucosidases were demonstrated in both the epithelial cytoplasm and the reticular cells of the lamina propria.	Not given		Few min	Not given	1 step to preserve; 6-7 for staining	Dry ice-acetone freezer, Harris-International cryostat, incubator, glass containers, microtome Various indoyl carbohydrate substrates, hematoxylin, eosin, alc, xylol, acetone, dry ice	Demonstration of intestinal glycosidases in the neonatal rat	Esterly (1967)
107. Leaves	Dried conifer needles	A method was given whereby thin sections of dry, fungus-infected conifer needles may be cut on a cryostat microtome at -15°C. Stained, temporary or permanent slides were made by this method using lactophenol-cotton blue or lactophenol-acid fuschin mount.	Permanent slides could be prepared by this method as well as temporary slides for diagnosis or photomicrography.	The method was varied slightly if the needle was badly infected. Also, different types of needles were handled with slight modifications.	2½ hr (unstained)	Not given	8-12, depending on staining technique	Cryostat microtome, glass dishes, glass slides, oven, dissecting needle, covers Aerosol OT (Carter Ink Co), LePage's mucilage, 4% formalin, Stanvis Nicoloidine soln, abs alc-ether mixt, 7% alc, periodic acid-Schiff (P.A.S.) stain	Mycological studies of infected conifer needles	Farris (1968)
108. Lichens	Growing lichens	_Usnea_ and _Lobaria_ lichens were kept on a moss bed under natural conditions (in an assimilation chamber?), and were exposed for 3-5 hr to every fluctuation of temp and light during each day of winter 1966-67. Photosynthesis and respiration were measured under various conditions to determine their sensitivity to cold in lichens.	_Usnea certaina_ and _U. submollis_ continued photosynthesis to -10°C while _Lobaria pulmonaria_ continued to -7°C. Respiration continued to -10°C and -12°C respectively.	Details of paper were not clear due to poor translation.	Plants were exposed 3-5 hr per day for one whole winter	Not given		Not clear	Study of photosynthesis and respiration of mountain lichens during exposure to cold	Atanasiu (1969)

FREEZING (Continued)

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109. Liver tissue	Mouse liver tissue slices	Mouse liver tissue was exposed to rapid and slow freezing and thawing, and to sonication to compare the effects on the activity of nuclear and mitochondrial enzymes. Nicotine adenine dinucleotide pyrophosphorylase, succinoxidase, succinic dehydrogenase, and glutamic dehydrogenase were the enzymes studied.	NAD-pyrophosphorylase was unaffected by any of the freezing procedures or by sonication. Succinoxidase activity was decreased 26-29% by fast freezing; more by other methods. Freezing on dry-ice gave the best preservation of succinic and glutamic dehydrogenases.	It appeared that many changes in enzyme activity were caused by disruption of the structure of mitochondria rather than denaturation of enzymic proteins.	15 min	Not given	Depends on method; 2-4	Microtome, beakers, Raytheon 10 KC magnetostriction oscillator, aluminum foil, refrigerator, tube filled with teflon pestal Dry ice, propane, isopentane, liq nitrogen, acetone, 0.25 M sucrose soln	Enzyme studies	Waravdekar (1964)
110. Liver tissue	Freshly excised rat liver	Considerable decreases in the sum of NADP + $NADPH_2$ conc in rat liver homogenates were occasionally observed during 30 min storage at 0°C. An attempt was made to find why these decreases occurred. Many substances were investigated to find a proper suspending soln. Also the effects of freezing on intact tissue and homogenates before extn of NADP and $NADPH_2$ were studied in detail.	Addition of 0.5 M nicotinamide + 5 mM tris buffer to 0.25 M sucrose for use as a suspending medium preserved NADP + $NADPH_2$ conc in homogenates at the same level as intact tissue for at least 30 min at 0°C. Freezing alone converts 30% of liver $NADPH_2$ into NADP in intact tissue.	A centrifugation process was described which cut processing of samples before anal down to approx 30-45 min. Preservation time was designed to coincide with this time span.		30 min	4	Containers, ultracentrifuge, scissors, Potter-Elvehjem homogenizer, ice bath, liq nitrogen container 0.5 M nicotinamide, 5 mM tris buffer, 0.25 M sucrose, plus many others	Anal of nicotinamide-adenine dinucleotides by the method of Slater, Sawyer and Straüli (1964)	Slater (1964)
111. Mitochondria	Isolated tomato tissue mitochondria	Isolated tomato tissue mitochondria were suspended in a reaction mixt of mannitol, $MgCl_2$, tris(hydroxymethyl)aminomethane, KH_2PO_4, and EDTA plus substrate and ADP. Samples of the suspension were diluted with 5% DMSO and frozen in a deep freeze (-18°C), over liq nitrogen vapors, or immersed in liq nitrogen.	Dimethyl sulfoxide prevented loss of respiratory control and decrease in efficiency of oxidative phosphorylation when plant mitochondria were stored in liq nitrogen. ADP did not stimulate inhibited respiration in mitochondria frozen without DMSO.	Isolated mitochondria provide a model system for studying the effects of freezing biol membranes.	Varies with method; <1 hr	4 wk	3-4	Polycarbonate tubes, glass vials, freezer, liq nitrogen freezer Mannitol, bovine serum, albumin, sodium barbital, $MgCl_2$, tris-(hydroxymethyl)aminomethane, KH_2PO_4, EDTA, DMSO, ADP, liq nitrogen	System for studying freezing effects on biol membranes	Dickinson (1967)

FREEZING (Continued)

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112. Muscle	Fresh squid muscle	Fresh squid muscle was frozen to -5°C and kept for 50 hr. 2 gm samples of the frozen muscle taken at intervals were ground in a mortar in 20 ml of 4% perchloric acid soln. The suspension was filtered, and the filtrate was analyzed for adenine and hypoxanthine nucleosides and nucleotides to assess the effects of freezing on these compounds.	During the first 10 hr a rapid dephosphorylation of adenosine triphosphate occurred with a concomitant accumulation of the diphosphate and of the monophosphate. The monophosphate accumulated gradually during 25 hr from the start while the diphosphate decreased. Adenosine monophosphate thus formed, without conversion to inosine monophosphate, was converted to inosine and then to hypoxanthine. These changes were almost quantitative.		3 hr	Sample was kept frozen 50 hr	2-3	Freezer, mortar and pestle, filter funnel 4% perchloric acid soln	Study of freezing effects on muscle	Saito (1958)
113. Muscle	Raw muscle between cover glasses with a copper constantan thermocouple embedded in it	Various coating agents including rough-surfaced vapor-nucleating substances (usually powd) and smooth-surfaced wetting agents were screened for their ability to increase rapid freezing of microscope slide prepn of muscle tissue in liq nitrogen. Cooling was recorded by a thermal junction at the center of the sample. Out of more than 100 substances ashes, kieselguhr, silicon oxide, phosphorus pentoxide, and Kleenex paper, as well as soap foams and a fluorocarbon compd were the best. Cooling velocity could be increased by 23:1.	Not given	Metal powd were not good coatings. No comment was made about the phys effects of freezing on the sample.	0.15-3.5 sec depending on coating used	Not given	3	Cover glasses, constantan thermocouple, liq nitrogen container Any one of several pulverized, granulated, or liq coating agents; liq nitrogen	Freezing of tissue sections and whole organs	Luyet (1961)
114. Muscle	Blocks of aged beef muscle	The effects of pre-treatment variables such as freezing rate and method of cutting on the acceptability of meat were investigated. Surface temp during freeze-drying and dehydration of meat <u>per se</u> were also studied.	High freezing rates (using liq nitrogen) impaired reconstitution and color while conventional freezing rates did not. Muscles cut transversely in the frozen or semi-frozen state were more acceptable than ones cut before freezing. Freeze-dried meat was tougher and less acceptable than frozen meat.		Varied with method	Up to 8 mon	3-4	Nitrogen spray freezer, ordinary meat freezer, meat cutter saw, freeze-dryer	Food	Bengtsson (1968)

FREEZING (Continued)

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115. Muscle	Freshly biopsied human muscle	Muscle biopsies, taken from adults after heavy exercise, were frozen immediately in liq nitrogen and kept at temp below -80°C. The samples were weighed at -20°C and deproteinized by placing them on top of 0.1-0.2 ml of frozen 3 M $HClO_4$ then thawing to 4°C. These samples were used to determine muscle tissue lactate after exercise.	None given		Few min	Not given		Liq nitrogen freezer, containers Liq nitrogen, 3 M $HClO_4$	Detn of muscle lactate by the modified fluorometric method of Lowry (1964)	Daimant (1968)
116. Muscle	Freshly excised frog sartorius muscle	An app (described) was designed to flatten a pair of muscles between two metal faces that had been cooled to -196°C in liq nitrogen. The muscles were simultaneously flattened and rapidly frozen.	Halving the thickness of a specimen quartered its freezing time. The authors estimated that the center of a squashed muscle reached -10°C in less than 100 msec.	Very thin specimens had a tendency to thaw on handling.	Few min	Not given	3-4	Special flattening and freezing app (described) Liq nitrogen	Investigation of chemical changes associated with muscle contraction	Kretzschmar (1969)
117. Muscle	Intact porcine muscle	A Freon cooled cryoprobe was described which allowed the metabolic activity of a tissue sample to be terminated before taking the tissue away from its blood supply. The app provided a large size sample and could be operated by personnel untrained in surgical techniques. Skeletal muscle metabolism was studied with the aid of this app.	Compared to freshly excised muscle, ATP and phosphocreatine levels were significantly higher in samples taken through cryobiopsy while lactic acid conc was lower. This indicated that conventional biopsy caused excision anoxia which altered metabolite levels, while cryobiopsy did not have this effect.	The probe with frozen tissue attached can be submerged in liq nitrogen and the frozen sample fragmented and powdered for extn or anal.	10 sec	Not given	4-5	Cryoprobe (described), liq nitrogen container Freon, liq nitrogen	Muscle sampling from live animals	Teeter (1969)
118. Muscle	Freshly excised taenia coli smooth muscle from guinea pigs	Strips of taenia coli smooth muscle from guinea pigs were bathed in oxygenated Kreb's soln with the DMSO conc gradually increased as the temp plateau was gradually decreased. Temperatures were chosen so that no ice crystals formed in the bathing soln or the muscle. Muscle activity was measured after the various exposures to DMSO at various temp.	20% DMSO in Kreb's soln did not affect muscle contractile response at 37°C. A lengthy discussion of DMSO toxicity at the other temp is given.		Varied		Varied	Test tubes, glass bubblers, cooling bath Kreb's soln, DMSO	Study of ice formation in, and DMSO effect on, smooth muscle	Elford (1970)

FREEZING (Continued)

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119. Mycoplasma	Agar block cultures	Agar blocks with heavy growth of human and rat strains of Mycoplasma and Serratia L. forms were frozen to -60°C.	Agar blocks frozen to -60°C for 6 mon produced viable organisms upon thawing.	The advantages of this method were that growth colonies could be seen on the agar block, and subculturing was not necessary.	Not given	6 mon	3	Agar blocks, freezer	Maintenance of viable strain cultures	Kundsin (1965)
120. Mycoplasma	Organisms suspended in appropriate medium	Mammalian and avian mycoplasmas were grown on a medium consisting of Difco PPLO broth, serum, yeast, thallium acetate, penicillin G, phenol red, and glucose, arginine, or urea until the color change was complete. Cultures were stored at -70°, -50°, and -30°C, or lyophilized in ampules contg either skim milk, aq dextrose-dextran, or bovine plasma albumin. They were sealed and stored at 4° and 37°C.	Organisms were viable after 2 yr at -70°, -50°, or -30°C in either vials or plastic trays. Samples lost less than 1 log_{10} viable organisms after 42 mon at -70°C. However, storage at -30°C resulted in a marked decrease in viable organisms and should be used for high initial titres only. Lyophilization resulted in loss of over 90% of the microorganisms, but remaining organisms were viable for 27-34 mon at 4° and at 37°C.	Stabilizing fluids used were not helpful in preservation.	Not given	42 mon at -70°C	Drying 4; lyophilization, 3-4	Pipettes, glass vials with screwcaps and rubber liners, incubator, plastic disposable trays, low temp refrigerator, plastic adhesive tape, drying oven, UV light irradiator, refrigerator (V.M. Berry, Liverpool), Petri dishes, glass ampules, freeze-dryer (Edwards High Vacuum) Skim milk, dextrose-dextran soln, bovine plasma albumin, Difco PPLO broth, serum, yeast, thallium acetate, penicillin G, phenol red, glucose, arginine, urea	Preservation of prototype strains	Addey (1970)
121. Mycoplasma	Cell suspension in culture medium	Cultured Mycoplasma cells were centrifuged, decanted, and resuspended in fresh, sterile broth culture medium to produce a conc of cells 30x greater than the original culture. Conc culture suspension was diluted 1:1 with 20% aq glycerol soln and hermetically sealed in ampules which were then cooled at 1°C/min to -40°C and stored in a liq nitrogen freezer at -150° to -196°C. Other samples of conc culture suspension were mixed with 24% aq sucrose soln, lyophilized, and stored under vacuum in sterile ampules at -70°C.	Liq nitrogen storage provided stable storage for some strains of Mycoplasma for as long as 9 yr. Lyophilization was also a successful technique if 12% sucrose soln was used as a protective additive.		20-22 hr for lyophilization	Up to 9 yr	Depends on method	Incubator, gyratory shaker, centrifuge bottles, ampules, freeze-dryer, centrifuge, cotton plugs, BF-3-2 freezer (Linde), liq nitrogen freezer, latex tubing, vacuum pump, heat sealer 12% (v/v) aq sucrose soln, appropriate culture media, liq nitrogen, dry ice-ethylene glycol-monoethyl ether mixt	Maintenance of type cultures	Norman (1970)

FREEZING (Continued)

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122. Nematodes	Live nematodes	Nematodes were treated with cyanide as an inhibitor 10 min prior to incubation in histochem reagents. The worms were then frozen rapidly to -35°C, slowly warmed for 10 min, and transferred immediately after thawing into incubation medium. To prepare sections, living nematodes were placed in Lipshaw M-1 Embedding Matrix and frozen directly onto the precooled object disc of a microtome.	Incubation temp was important to the color reaction in stained specimens. Color deposits were pale at 19°C and strong at 28°C. Sections 12 µ thick and covering 70% of the nematode length could be cut by this method.	Staining techniques were discussed for identifying various enzyme systems especially cytochrome oxidase.	Few min	Not given	4-5	Containers, freezer, water bath, microtome, incubator Cyanide, incubating medium, Lipshaw M-1 Embedding Matrix, basal culture medium supplemented with 10% liver ext	Histochem demonstration of cytochrome oxidase	Deubert (1968)
123. Nematodes	Nematode suspensions in culture media	Free-living nematodes were successfully frozen in liq nitrogen in heat-sealed glass ampules using 5 or 10% DMSO as a protectant. Thawed samples were resuspended in 2 ml of Heller's soln with added ergosterol and incubated. Active nematodes were counted on the 4th day after thawing.	From 18-87% recovery of the worms occurred upon incubation for 4 days after thawing. The number of viable worms was about the same after 6 mon storage as it was after freezing and thawing immediately.		2-3 hr	6 mon	6-7	Culture plates, Petri dishes, centrifuge, water bath, vials or ampules, heat sealer, cotton, styrofoam cylinder, Programmed Temp Controller (Canalco Co), liq nitrogen bath Distd water, DMSO, liq nitrogen, modified Heller's soln, ergosterol	Type cultures for nematodes	Hwang (1970)
124. Nerves	Freshly isolated frog sciatic nerve	Frog sciatic nerves with or without perineural sheaths were treated with various conc of DMSO or glycerol in amphibian Ringer's soln. Some samples were frozen to -10°C before treatment, some were frozen after, and others were not frozen at all, thus allowing a distinction to be made between drug toxicity and freeze-thaw damage.	Neither glycerol nor DMSO severely damaged nerves, but some effects were noted. DMSO was less damaging than glycerol and was almost independent of the nerve sheath. Glycerol changes were greater in desheathed nerves. DMSO protected desheathed nerves from freeze-thaw damage, but glycerol did not.	Conduction velocity, action potential, and absolute refractory period were measured as electrical parameters of nerves.	Not given	Not given	4-5	Glass containers DMSO, glycerol, amphibian Ringer's soln	Effects of cold on nerve preservation	Pribor (1969)

FREEZING (Continued)

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125. Nucleoside diphosphatase	Purified rat-liver nucleoside diphosphatase	Nucleoside diphosphatase was purified from ext of rat-liver acetone powd by the method of Schramm and Morrison (1968) and stored for 6 mon at -10°C before use. The effect of storage on kinetics and reaction mechanism of the enzyme was investigated.	Mol wt and reaction to $MgATP^{2-}$ remained the same for stored enzyme as for fresh enzyme. The stored enzyme gave a linear plot of 1/v vs 1/magnesium inosine diphosphate conc, and its max velocity was unaffected by the addition of modifier. A theory of the reaction mechanism for the stored enzyme is also given.		Not given	6 mon	Not all given	Freezer	Study of the effects of ageing on the kinetics and reaction mechanism of nucleoside diphosphatase	Schramm (1970)
126. Pepsin	Standard soln of commercial porcine pepsin adjusted to pH 1.8	Commercial crystalline porcine pepsin was made into a stock soln contg 5 or 10 μg of pepsin/ml of 0.024 M HCl at pH 1.8. To these soln were added acidified glycerol and/or acidified canine or bovine albumin. Samples were kept at room temp or cooled to 4°, 0°, -4°, or -20°, and the temp effects on pepsin activity were measured.	The stock pepsin soln maintained 100% of its activity at 0°C for up to 5 hr without a preservative. 100% pepsin activity was maintained for 6 days at -20°C in the presence of albumin and glycerol. 80-90% pepsin activity remained after 20 days in the presence of albumin and glycerol.	Preservatives were necessary for prolonged storage of sample.	Varied	5 hr with no preservative; up to 20 days with a preservative with some loss of activity	3	Ice bath or freezer, storage containers HCl, glycerol, pepsin, albumin	Gastric secretion 'standard'	Hunner (1969)
127. Phosvitin	Isolated phosvitin protein	If an aq soln of largely disordered phosvitin protein is frozen under certain conditions, subsequent thawing produces a soln which contains phosvitin in a folded conformation. This 'structure-making' effect of freezing was investigated especially with regard to its relationship to denaturation of proteins.	Phosvitin changed from unordered to B structure upon freezing and thawing. It occurred at pH 2 or less but was enhanced by acidity. Increases in salt or protein conc suppressed the phenomena, and the ordered structure was heat labile. Aggregation and polymerization were essential, and the phenomena seemed to be linked to freezing rather than thawing.	The authors hypothesized that as acid was excluded from the growing ice upon freezing, the acid 'tide' just ahead of the liquid-ice interface caused the transconformation followed by the trapping of the ordered protein in the rigid ice.	Not given	Samples were rethawed after a short time		Platinum-coated base test tubes, ethanol-dry ice bath, teflon cylinder	Study of protein denaturation and freezing and thawing phenomena	Taborsky (1970)

FREEZING (Continued)

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128. Phycoerythrin	Phycoerythrin in 0.001 M phosphate buffer, pH 6.6	Purified phycoerythrin was obtained from Porphyridium cruentum cell suspensions. Samples of the chromoprotein in 0.001 M phosphate buffer, pH 6.6, were frozen rapidly to -20°, -30°, -45°, -60°, or -76°C, held for 10 min, then warmed rapidly or slowly to room temp. Rates of cooling and thawing varied.	Absorption spectrum, fluorescence yield, sedimentation, and electrophoretic properties of phycoerythrin are all altered by freezing and thawing. A discussion of the nature and significance of these changes was given.		Varied with method	Not given	2-3	Centrifuge, dialyzer, Aminco-French pressure cell, refrigerator, test tubes, controlled heating and cooling baths 0.01 M phosphate buffer, pH 6.6; 0.001 M phosphate buffer, pH 6.6; n-butanol; 20% $(NH_4)_2SO_4$ soln	Study of storage temp effects on chromo-proteins	Leibo (1964)
129. Saliva	Fresh expectorated human saliva	Volunteers expectorated into a sterile container before eating, smoking, or brushing their teeth in the morning. This sample was immediately frozen to -78.5°C by placing it in a Dewar flask filled with dry ice. The sample was kept in this state until ready for anal.	Samples were kept 24 hr before anal and could probably be kept much longer.		Few min	24 hr	4(incl sterilization)	Dewar flask, 20 ml sterile injection vials, autoclave, drying oven, autoclave foil Dry ice	Gas chromatographic anal of saliva volatiles	Larsson (1969)
130. Saliva	24 hr human sputum sample	24 hr collections of sputum were made from each donor for a mon. Within 12 hr of collection the sol phases were separated by centrifugation. Sol phases were pooled, divided into small amt, and stored at -70°C.	Samples stored in this manner were suitable for electrophoretic studies.		Few hr	Not given	4-5	Containers, centrifuge, freezer	Sputum electrophoresis and anal	Ryley (1970)
131. Skin	Strips of undried rabbit skin	Large strips of rabbit skin were stretched on a cheesecloth pad, then immersed in liq nitrogen. Some of the strips were immersed in vaseline oil or glycerine for protection before freezing.	Skin specimens stored up to one mon were used for autografts and homografts; however, homografts were eventually rejected. Vaseline oil improved the viability of the skin specimens somewhat.	Specimens should not be dipped into liq as liq may cause ice which can rupture the cells.	10 min	1 mon optimum; up to 7 mon max	3	Cheese cloth, liq nitrogen container, nitrogen tank	Skin grafts	Lapchinsky (1962)

FREEZING (Continued)

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132. Skin	Freshly excised rat skin	Abdominal skin from adult rats was excised, and muscle and subcutaneous tissue was removed. The skin samples were placed in tubes contg 3 cc of various media, sealed with cotton plugs and adhesive tape, and stored at 4^{o}-$7^{o}C$ for various lengths of time after which the samples were reimplanted. Other samples were sealed in ampules with thermocouples attached to the graft. The rate of freezing and thawing was varied, and its effect on the samples observed before and after reimplantation.	Refrigerated samples in 5% glycerol soln in 0.85% NaCl could be stored up to 14 days and still produce permanent survival on reimplantation. Samples were protected against freezing and thawing damage by either 10% glycerol or 10% DMSO in 0.85% saline soln.	Thawing rate was more important to survival than freezing rate, and rapid thawing was essential.	15 min	Up to 14 days	5-6	Surgical tools, tubes, cotton plugs, adhesive tape, ampules, thermocouples, refrigerator, water bath, special freezing app Various media contg glycerol, DMSO, serum, 0.85% saline soln, dry ice, acetone	Skin grafts	Lehr (1964)
133. Skin	Freshly excised mouse skin	Mouse skin, full-thickness autografts were pretreated for 1 hr in 5% or 15% solutions of glycerol or DMSO in Ringer's soln. Samples were then frozen to $-75^{o}C$ in Stender dishes on dry ice at an av rate of $1.3^{o}C$/min. After thawing, the sections were transplanted and observed for hair color, direction of hair growth, and microscopic appearance.	5% levels of either DMSO or glycerol were less toxic and gave the greatest postthaw survival. The pretreatment had more effect on postthaw viability than the actual freezing and thawing. Glycerol and DMSO were approx eq in toxicity and cryoprotection.			Samples were thawed immediately and transplanted	3	Surgical tools, Stender dishes 5 or 15% glycerol in Ringer's soln, 5 or 15% DMSO in Ringer's soln, solid CO_2	Transplantation	Sherman (1965)
134. Skin	Freshly excised human abdominal skin	Small pieces of human skin were placed in Eagle's medium in Earle's balanced salt soln plus 10% calf serum and varying amt of either glycerol or DMSO. After 1-8 hr the pieces were placed in finger cots with more medium. The cots were put in a rubber glove, and the glove was frozen at 1^{o}-$8^{o}C$/min in liq nitrogen. Effects of conc of cryoprotectant and rate of cooling and heating on epithelial growth were investigated.	Human skin pretreated with 20-30% glycerol at $4^{o}C$ for 1-2 hr before freezing did not show epithelial damage, and it grew on plasma clot culture like fresh skin.	Hyaluronidase did not facilitate penetration of glycerol into skin.	2-3 hr (optimum)	Not given	5	Petri dishes, surgical tools, sterile finger cots, surgical gloves, Linde BF-1 freezer Eagle's medium, Earle's balanced salt soln, DMSO, glycerol, liq nitrogen, hyaluronidase	Skin tissue culture	Athreya (1969a)

FREEZING (Continued)

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135. Sodium methicillin	Sodium methicillin reconstituted with water	Vials of sodium methicillin USP for injection were reconstituted with sterile water and stored at -16°C in a freezer. Samples were also stored at 24°C and 5°C, and others were incubated, then frozen. Potency of the various soln was tested.	Reconstituted sodium methicillin was potent for 71 days after storage at -16°C.	Frozen samples that were thawed, stored at 4°C, and refrozen lost some potency.	Approx 1 hr	> 71 days	2	Freezer, disposable hypodermic syringe Sterile water	Clinical use	Stolar (1968)
136. Spermatozoa	Fresh human and fowl ejaculate	Human spermatozoa were successfully protected from freezing effects by addition of 5% glycerol (equal parts of semen and 10% glycerol in Baker's fluid). Ethylene glycol and propylene glycol gave similar results. Fowl spermatozoa required 40% glycerol for protection against freeze-drying and freezing at -79°C.	Motile human and fowl spermatozoa were observed after freezing and thawing at -79°C in the presence of glycerol. Motile fowl spermatozoa could be observed also after freeze-drying in the presence of glycerol.	Revival was much better when semen was frozen in bulk rather than in minimal amt in capillary tubes.	Varies with method	Samples were thawed or reconstituted	Depends on method	Freezer, containers Glycerol, Ringer's soln	Artificial insemination	Polge (1949)
137. Spermatozoa	Fresh ram and bull ejaculate	Ram and bull semen were collected, and the initial pH was maintained with phosphate buffers and citrate-egg yolk medium. The semen was diluted 1:4 with egg yolk medium at 30°C and cooled slowly over 2-3 hr to 5°C. Aliquots were taken at 29°, 17° and 5°C and diluted with equal vol of the appropriate glycerol and sugar medium to give either 3.75% or 7.50% (v/v) glycerol and 1.25% fructose. After each dilution at higher temp, cooling was continued to 5°C. After 1 hr at 5°C, samples were slowly frozen to -79°C. Samples were thawed after 24 hr, and sperm motility was measured.	Ram and bull spermatozoa frozen in glycerol-phosphate media survived best at a pH near neutrality and at a glycerol conc of 3.75% and 7.5%. Isosmatic media were most suitable for freezing spermatozoa.		Several hr	Not given	6-7	Electrical stimulator, artificial vagina, containers, refrigerator, pipettes, freezer Phosphate buffers, egg yolk-citrate medium, glycerol, fructose	Artificial insemination	Blackshaw (1960)

FREEZING (Continued)

SPECIMEN DESCRIPTION	ORIGINAL FORM OF SAMPLE	BRIEF DESCRIPTION OF METHOD	LIMITATIONS OF METHOD	OTHER COMMENTS ABOUT METHOD	ESTIMATED PRESERVATION TIME	SURVIVAL TIME	NO. OF STEPS	EQUIPMENT AND REAGENTS USED	END USE OF SPECIMEN	REFERENCE
138. Spermatozoa	Fresh bull ejaculate	Samples of bull semen with high initial motility were diluted at 30°C with 9 vol of one of several diluents plus added non-dialysable milk solids, casein, or other proteins. The mixtures were chilled slowly to 5°C over 2 hr. Equal vol of precooled 15% glycerol-85% diluent were added to the chilled suspensions to give a final glycerol conc of 7.5%. The mixt were allowed to equilibrate for 18 hr with glycerol before being sealed in ampules and cooled to -79°C. After 24 hr, ampules were thawed and incubated, and motility and % motile sperm were estimated. Diluents used were: 0.154 M sodium chloride; 0.77 M sodium chloride-0.154 M fructose; and Ringers soln-fructose.	Non-dialysable milk solids and casein both improved the revival of deep frozen bull spermatozoa. Addition of potassium, magnesium and calcium chlorides or sodium citrate to the diluents did not significantly improve the revival of the deep-frozen semen, in fact levels of potassium similar to those in milk were detrimental to revival. Addition of fructose after chilling improved revival, even when fructose was already present in the diluent.		2½-3 days	Not given	Varied with expt	Artificial vagina, containers, refrigerator, ampules, freezer, incubator, dialysis app Glycerol, various diluents (described), non-dialysable milk solids, casein, fructose, potassium, magnesium, calcium chloride, and lactose	Artificial insemination	Choong (1963)
139. Spermatozoa	Fresh ejaculate from bull, boar, and turkey	Spermatozoa were removed from the extracellular fluid by placing the sperm suspension in a glass tube that contained 4 cm of powd cellulose on top of fine glass wool. The fluid was drawn by negative pressure through the tube, leaving the cells trapped in the cellulose. The extracellular fluid was centrifuged, and the supernatant was frozen in liq nitrogen. Anal of extracellular fluid of boar, bull, and turkey semen, before and after freezing, were conducted to test for leakage of various components into the extracellular fluid due to handling and freezing. The effects of various extenders and cryoprotectants on cell leakage were also studied.	Leakage of cellular components into extracellular medium could provide a possible test of spermatozoan cell damage. However, before this principle can be applied better techniques for separation of spermatozoa from medium are needed. Data presented indicated that cellular damage occurred prior to freezing as well as during freezing.		Varied	Most samples were thawed within 2 wk	5-6	Tapered glass tubes, powd cellulose, glass wool, vacuum, demineralized and deionized water, drying oven, centrifuge, liq nitrogen freezer Glycerol, ethylene glycol, 1,3-propanediol, DMSO	Study of biochemical changes during storage of sperm	Graham (1967)

FREEZING (Continued)

SPECIMEN DESCRIPTION	ORIGINAL FORM OF SAMPLE	BRIEF DESCRIPTION OF METHOD	LIMITATIONS OF METHOD	OTHER COMMENTS ABOUT METHOD	ESTIMATED PRESERVATION TIME	SURVIVAL TIME	NO. OF STEPS	EQUIPMENT AND REAGENTS USED	END USE OF SPECIMEN	REFERENCE
140. Spermatozoa	Fresh human ejaculate	Semen specimens from several human donors were mixed 1:1 (v/v) with egg yolk-glycerol-citrate medium and refrigerated in ampules at 6°C for 7 days, or stored in liq nitrogen for 2-75 wk. DNA content of the stored specimens was examined and compared with the DNA content of fresh specimens.	DNA remained constant after refrigeration and after freezing for all of the time periods studied.		Varied with method	7 days at 6°C, 75 wk at -96°C	4-5	Glass ampules, refrigerator, freezer, liq nitrogen freezer Egg yolk-glycerol-citrate medium	DNA anal	Ackerman (1968)
141. Spermatozoa	Fresh bull semen	Beta- or alpha-amylase was dissolved in 0.9% sodium chloride, 0.2 or 2.0 mg/ml, within 2 hr before semen was extended. One ml of amylase soln was added to each 99 ml of egg yolk-sodium citrate dihydrate extender. The non-glycerol extenders contg amylases at levels of 0, 2, or 20 μg/ml were added to 200 bull semen ejacula. The semen samples were then glycerolized so that the final conc of amylase were 0, 1, or 10 μg/ml. The semen was frozen and stored at -195°C for 2 wk-4 mon before thawing in 5°C water for insemination.	Cows inseminated with amylase-treated semen had a 70.7% av fertility out of 17,597 cows, whereas controls had 68.1% av fertility out of 4,294 cows. Both alpha- and beta-amylase gave comparable results using 10 μg/ml of extended semen.	Amylase added to extended bovine semen may bring about fertilization of ova without prolonged residence of the sperm in the female tract.	1-2 hr	2 wk-4 mon	6-7	Containers, liq nitrogen freezer Beta-amylase, alpha-amylase, sodium chloride, egg yolk-citrate extender	Artificial insemination	Kirton (1968)
142. Spermatozoa	Fresh bull semen	Bull spermatozoa in various buffered egg-yolk media, and in skim milk, were exposed to several levels of glycerol at 5°C for 6 hr, 30 min, and 10 sec prior to freezing. Freezing was done by placing droplets of the extended semen in small indentations on blocks of dry ice. Results were compared to find the optimum exposure of sperm to glycerol before freezing.	Skim-milk extender was not suitable for the rapid freezing of sperm by the pellet method. The 10 sec exposure time to glycerol gave the best sperm motility after freezing in comparison with 30 min or 6 hr exposures. Glycerol levels between 4.5% and 9.5% gave the highest postthaw survival rate for sperm. Motility following thawing was also affected by the thawing medium.		1-2 hr	Samples were thawed after 1 wk	> 10	Tubes, water bath, polyethylene syringes, dry-ice block, refrigerator, liq nitrogen freezer Glycerol, Tris extender, skim milk, egg yolk-citrate extender	Artificial insemination	Berndtson (1969)

FREEZING (Continued)

SPECIMEN DESCRIPTION	ORIGINAL FORM OF SAMPLE	BRIEF DESCRIPTION OF METHOD	LIMITATIONS OF METHOD	OTHER COMMENTS ABOUT METHOD	ESTIMATED PRESERVATION TIME	SURVIVAL TIME	NO. OF STEPS	EQUIPMENT AND REAGENTS USED	END USE OF SPECIMEN	REFERENCE
143. Spermatozoa	Fresh boar semen	Four freezing methods were tried on boar semen: pellet freeze, nitrogen vapor freeze, fast mechanical alc freeze, and slow mechanical alc freeze. Glycerol and NN-dimethylacetamide were used as cryoprotectants. The methods and protective agents were compared for effectiveness in sperm preservation.	The slow mechanical alc freezing method gave the highest spermatozoa recovery rates. Glycerol protection produced higher recovery rates than NN-dimethylacetamide, but a combination of the two appeared to give good recovery rates also. However, no fertility occurred following insemination of 14 animals with frozen semen contg both glycerol and NN-dimethylacetamide as protective agents. No difference was noted between different types of freezing containers.		Several hr	< 3 hr after thawing	Varied with expt	Refrigerator, containers, glass ampules, plastic straws, liq nitrogen storage tank, dry ice blocks, alc freezing bath, liq nitrogen freezer Skim milk-egg yolk-glucose extender, liq nitrogen vapor	Artificial insemination	Dalrymple (1969)
144. Spermatozoa	Fresh human ejaculate	Human semen specimens were obtained by ejaculation and allowed to liquify. Samples were analyzed for motility, conc, morphology, and sperm penetration. Suitable samples were mixed with a glycerol-egg yolk protective mixt in a 1:1 ratio at room temp. The semen-media mixt was then drawn into plastic straws, and the straws were sealed and placed in test tubes contg 95% alc. The tubes were cooled to 4°C, then to -40°C and were finally stored at -196°C.	3 out of 7 patients had become pregnant using stored semen. The semen had a postthaw motility rate of 25-45%.	8-10 straws full of semen-media mixt were obtained for each ejaculate.	1 hr	Up to 180 days	8-9	Water bath, plastic straws, moisture solidifying powd, test tubes, alc bath, beakers, freezer, Linde LR-35 liq nitrogen freezer Protective media contg egg yolk, 15% glycerol, and glucose in water (shelf life 3 mon at 4°C); sodium citrate; glycine-erythromycin mixt; 95% ethyl alc	Artificial insemination	Matheson (1969)
145. Spermatozoa	Fresh rabbit semen	Rabbit semen samples with good initial motility and density were frozen in sealed glass vials after the addition of 9 vol of milk-diluent contg varying amt of DMSO. Diluted semen was cooled from room temp to 5°C over approx 2 hr. The vials were then frozen to -79°C and stored overnight in solid carbon dioxide. After thawing, the samples were examined for motility and tested for fertility.	Samples of rabbit semen frozen in 14% DMSO (v/v) in a skim milk diluent contg fructose, which were cooled to 5°C in an isotonic diluent then frozen in a hypertonic diluent, had optimum postthaw motility. 46% of ova fertilized with sperm which had been frozen for 6 mon developed into embryos.		Several hr	Up to 6 mon	4	Glass vials, artificial vagina, osmometer, pipettes, rubber caps, refrigerator, freezer Penicillin, streptomycin, skim milk diluent, DMSO, fructose, solid carbon dioxide	Artificial insemination	O'Shea (1969)

FREEZING (Continued)

SPECIMEN DESCRIPTION	ORIGINAL FORM OF SAMPLE	BRIEF DESCRIPTION OF METHOD	LIMITATIONS OF METHOD	OTHER COMMENTS ABOUT METHOD	ESTIMATED PRESERVATION TIME	SURVIVAL TIME	NO. OF STEPS	EQUIPMENT AND REAGENTS USED	END USE OF SPECIMEN	REFERENCE
146. Spermatozoa	Fresh human ejaculate	Human semen samples were allowed to liquefy at room temp for 30-60 min. The vol was measured, and a sperm count was made. At varying times before freezing, glycerol was added dropwise to each specimen and gently swirled until a final conc of 7.5% was reached. The specimens were placed in ampules, sealed, and frozen by the fast- or slow-freeze method. Later the ampules were thawed in a 37°C water bath and the contents evaluated.	Max survival was found when sperm were thawed before 24 hr or after 5 wk of storage. Thawing in the interim time produced lower survival rates. The best recovery of motility, 35.3%, occurred when samples were incubated with 7.5% glycerol (final conc) for 6 min. The fast-freeze method (holding the ampule over nitrogen vapor) gave a post-thaw motility of 44% compared to 21% for the slow-freeze method. Pregancies have been reported from sperm stored up to 6 mon by this method.	The same method was applied to orangutan primates with some success; however, the method needs modification for successful application to primates other than man.	Up to several hr	6 mon	7-8	Containers, slide warmer, slides, sperm counter, pipettes, ampules, heat sealer, freezer, water bath Glycerol, liq nitrogen	Artificial insemination	Trelford (1969)
147. Spermatozoa	Freshly collected fish sperm	Sperm from sexually mature walleye were collected in vials. The vials were capped, placed in a styrofoam container of crushed ice, and transported to a laboratory. Here 2 ml aliquots were frozen at 0°C, while other samples were collected into blood capillary tubes and centrifuged to give spermatocrit values. The rest of the semen was centrifuged, and the decanted seminal plasma and drained packed cells were each frozen separately. Sperm cell cytoplasm was prepared from the frozen packed cells. Seminal plasma was also prepared by centrifugation of the previously frozen aliquots. Pre- and post-freezing levels of several constituents of seminal plasma and sperm cell cytoplasm were analyzed and compared.	Freezing caused a 1/3 reduction in vol of seminal plasma. Chloride conc in seminal plasma remained about the same, while sodium and calcium declined, and magnesium, phosphorus, potassium, protein, urea nitrogen and uric acid increased. The freezing point depression of the plasma increased from -.489 to -.635 following freezing. These changes are attributed to cold shock and suggest a change in the selective permeability of the sperm cell membrane.	Fish sperm preservation was hampered by a lack of basic knowledge of the biochem and physiol of fish semen.	Not clear	Not given	9-10	Gill nets, glass vials, styrofoam transport container, refrigerator, blood capillary tubes, centrifuge Physiol saline soln	Anal of the chem and phys properties of walleye sperm	Gregory (1970)

FREEZING (Continued)

SPECIMEN DESCRIPTION	ORIGINAL FORM OF SAMPLE	BRIEF DESCRIPTION OF METHOD	LIMITATIONS OF METHOD	OTHER COMMENTS ABOUT METHOD	ESTIMATED PRESERVATION TIME	SURVIVAL TIME	NO. OF STEPS	EQUIPMENT AND REAGENTS USED	END USE OF SPECIMEN	REFERENCE
148. Spermatozoa	Fresh ram semen	Ram semen diluted with egg-yolk citrate and glycerol was cooled to 5°C in 2½ hr and divided into 4 aliquots: one served as a control; one was incubated at 38°C for 1 hr; another was frozen at 1°C/min from 5° to -10°C, then 2°/min from -10° to -18°C, then 4°/min from -18° to -35°C, and finally at 5°/min from -35°C to -70°C with no incubation; and the final aliquot was frozen and incubated at 38°C for 1 hr. After thawing the samples were separated into seminal plasma and sperm pellet, and the sperm pellet was sonicated to release the sperm cell contents. Following these treatments both seminal plasma and sperm from each aliquot were assayed for acid phosphatase, lactic dehydrogenase, and DNA.	Freezing and thawing produced a significant increase in the acid phosphatase band $P\text{-}AP_2$ of seminal plasma and a decrease in the spermatozoal acid phosphatase, suggesting that the enzyme leaked out of the sperm into the diluent. The three lactate dehydrogenase isoenzymes measured did not change their activity following freezing, thawing, and incubation. DNA content of ram spermatozoa also remained unchanged.		3-4 hr	Not given	7-8	Containers, refrigerator, pipettes, incubator, freezer, centrifuge, ice bath, beakers Biosonik sonicator, ethanol bath Egg yolk-citrate extender, glycerol, sucrose-EDTA soln	Study of freezing and thawing effects on ram semen	Nath (1970)
149. Spermatozoa	Fresh ram semen	Expt were conducted to examine the survival of ram spermatozoa frozen in the pellet form at various temp below -79°C. Aliquots of pooled semen were diluted 1:4 at 30°C with a variety of glycerol-contg diluents such as: raffinose-citrate, fructose, glucose, lactose, or raffinose. Each diluent also contained 15% (v/v) egg yolk, and the final glycerol level was 2-4%. Diluted samples were dropped onto a steel plate, with small indentations, that had previously been cooled by placing on a block of dry ice and lowering both block and plate into liq nitrogen. After 3-4 min frozen semen pellets formed, and these were then transferred into liq nitrogen for storage. A variety of cooling temp and rates were investigated.	Egg yolk-raffinose-citrate, yolk-raffinose, and yolk-lactose, all contg 4% glycerol, gave equal protection to ram spermatozoa during freezing. These three diluents were better than the others tried. Pelleting on either dry ice or on the steel plate in a range of -100° to -160°C gave better results than freezing at -180°C on both block and plate together or pelleting directly into liq nitrogen. Storage for 1-4 hr at 5°C prior to freezing, the presence or absence of egg yolk, and thawing temp of 37° or 45°C did not affect the spermatozoa survival rate significantly. Overall survival of spermatozoa was in the 30-40% range.	A poor survival rate was observed for ram semen that was pelleted directly into liq nitrogen.	Varied with method; generally several hr including thawing	Not given	6-8	Artificial vagina, flasks, pipettes, refrigerator, Fiske cryoscope, stainless steel plate (described), Linde LD-40 freezer, dry ice blocks, thermocouples, multichannel recorder, incubator Glycerol; egg-yolk; raffinose-citrate, glucose, lactose, fructose and raffinose diluents; liq nitrogen; glucose-citrate thawing soln	Artificial insemination	Salamon (1970)

FREEZING (Continued)

SPECIMEN DESCRIPTION	ORIGINAL FORM OF SAMPLE	BRIEF DESCRIPTION OF METHOD	LIMITATIONS OF METHOD	OTHER COMMENTS ABOUT METHOD	ESTIMATED PRESERVATION TIME	SURVIVAL TIME	NO. OF STEPS	EQUIPMENT AND REAGENTS USED	END USE OF SPECIMEN	REFERENCE
150. Spermatozoa	Fresh bull ejaculate	Several expt were conducted to determine the optimum levels of DMSO and glycerol in homogenized milk, skim milk, or egg yolk-Tris extenders for preservation of bovine semen. Samples were precooled 1-4 hr. All samples were glycerolated in 4 eq parts at 15 min intervals, and then placed in hermetically-sealed ampules and frozen in a vapor phase canister according to the method of Benson (1968).	In no case did the addition of DMSO to either milk extender result in a higher mean motility than that obtained with the optimum level of 6% glycerol alone. However, DMSO in combination with 6% glycerol in egg yolk-Tris extender gave a significant increase in post-freeze spermatozoa motility. Optimum prefreeze cooling time was 1 hr.		Several hr	Not given	6-7	Artificial vagina, refrigerator, flasks or tubes, glass ampules, sealer, vapor phase liq nitrogen canister Glycerol, homogenized milk extender, skim milk extender, egg yolk-Tris extender, streptomycin, penicillin, DMSO	Artificial insemination	Snedeker (1970)
151. Stem rust uredospores	Ungerminated spores	The following methods were compared for storing uredospores of stem rust: 1) Spore moisture was reduced to about 10%, and spores were stored at 4°C; 2) Spores were vacuum dried and stored in the absence of oxygen and water vapor; 3) Spores were stored in liq nitrogen at -196°C.	Spores could be stored 1-2 yr at 4°C if spore moisture was reduced to about 10%. Vacuum-dried spores could be stored up to 5 yr in the absence of oxygen and water vapor. Spores frozen in liq nitrogen could remain viable indefinitely.	Rate of cooling and thawing was not critical for survival of frozen uredospores, nor was the moisture content below 25%. Spores frozen to -196°C require heat shock at 40°C to restore max germinability.	Differs with method	1-5 yr	Differs with method; from 3-5	Vacuum dessicator or oven, liq nitrogen freezer, refrigerator, infected stems, collection and storage containers Water-agar culture, liq nitrogen	Maintenance of viable, exptl inoculum; epidemiology studies	Bromfield (1967)
152. Streptococcus lactis	Cell culture suspension	Streptococcus lactis was stored at 3°C, -20°C, and -196°C. Effects of storage at different temp on acid production, viability, and proteinase activity and structure were investigated.	Cultures of Streptococcus lactis frozen rapidly to -196°C showed no loss in proteolytic activity or acid production on milk, after 60 days storage. Storage at 3°C or -20°C impaired both factors.	Proteinase activity is important in the ability of stored cells to grow and perform normally in milk following storage whether storage be at 3°C, -20°C, or -196°C.	< 2 hr; depends on method	Up to 60 days	Not clear	Refrigerator, deep freeze, nitrogen freezer, centrifuge, containers Culture media, liq nitrogen	Effect of temp on proteinase structure and activity	Cowman (1969)
153. Sucrose gradients	Layered sucrose solutions	Beginning with the lightest sucrose soln, the aliquots of increasing sucrose conc were layered into Beckman cellulose nitrate centrifuge tubes with a Brakke funnel. Overnight diffusion produced smooth gradients. The gradients were then frozen at -60°C and later thawed to 25°C by placing the tubes in a test tube rack and letting the air thaw them. Thawing took approx 90 min.	Density gradient centrifugations are inconvenient because small numbers of fresh gradients must be made before each expt. This method allows the gradients of sucrose to be frozen for a convenient steady supply. Each gradient is virtually identical to the other.	The method did not work for NaCl gradients because the frozen saline expanded and split the nitrocellulose tubes. However, it could be used for salt-contg sucrose gradients with certain limitations.	1 day	Not given	4-5	Beckman cellulose nitrate centrifuge tubes, Brakke funnel, test tube rack, pipettes, freezer 0-40% (w/w) sucrose, in 5% increments; or 0-60% (w/w) sucrose, in 10% increments	For use in density gradient centrifugation	Shore (1969)

FREEZING (Continued)

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154. Tendon tissue	Small pieces of freshly excised tendon	Tendons were preserved by 5 different methods: 1) Small pieces of tendon were stored in Kovalenko's glucose-citrate-penicillin soln at 4°C, 2) Tendon pieces were frozen at -25°C, 3) Tendon pieces were soaked in 15% glycerine soln, then frozen at -25°C, 4) Specimens were placed in 15% glycerine, then frozen at -183°C and stored at -25°C, 5) Specimens were lyophilized. Tissue cultures of all samples were attempted as a test of viability of the tissue.	Cells of samples treated with glycerine and frozen at -25° or -183°C could be cultured up to 3 mon after thawing. Those cooled at 4°C in glucose-citrate-penicillin soln could be cultured up to 18 days. Lyophilized tendon tissue showed no cell growth.	The presence of glycerine for protection against cell injury during freezing was essential to cell viability.	Differs with method	18-90 days depending on method	Varies with method; generally 3-5	Freezer, refrigerator, lyophilizer, containers, surgical tools, vacuum dessicator or oven 15% glycerine, Kovalenko's glucose-citrate-penicillin soln	Tendon transplant	Demichev (1969)
155. Thyroid glands	Fresh beef thyroid glands	Fresh beef thyroid glands from a slaughter house were immediately wrapped in plastic bags, frozen, and stored at -25°C. The effect of freezing on microsomal peroxidase was investigated.	Frozen thyroid tissue stored in plastic bags could be kept for 1 yr with no loss in peroxidase activity. However, a 50% loss of activity occurred within a few wk if the plastic bags were omitted.		Few min	1 yr at -25°C	3	Plastic bags, freezer	Isolation and identification of peroxidase	Ljunggren (1968)
156. Tissue culture cells	Cell suspensions	Ampules of tissue cells in 10% DMSO with 15% calf serum in Gey's salt soln were slowly frozen to -196°C (1°C/min to -25°C; then more rapidly) in a container over liq nitrogen. Continous cell lines frozen by this technique included several HeLa strains and MK_2. Primary cultures included human amnion, human embryo kidney and lung, and monkey kidney.	Unlimited storage could be maintained as long as cells were frozen slowly and maintained at liq nitrogen temp.	The nitrogen container can be loaded before hand, and it will last from 7-90 days without reloading.	4-6 hr	Unlimited if temp is maintained	4	Linde low-temp storage containers, ampules, Dewar-polystyrene freezer Liq nitrogen, DMSO, 15% calf serum in Gey's salt soln	Maintenance of cell lines	Greaves (1963)
157. Tissue culture cells	Cells suspended in Nagle's medium	Cat kidney cells, L-cells, and HeLa cells were grown in protein-free Nagle's medium and pooled. 12 ml samples were centrifuged, and supernatants were replaced with a medium contg DMSO at various conc. 1 ml aliquots were sealed in ampules, then placed at 4°C for 20-30 min. Finally they were slowly frozen to -135°C.	Cell viability was 86-90% for L-cells stored in 4% DMSO for 1 mon; 81-87% for HeLa stored in 8% DMSO; and 74-86% for cat kidney cells stored in 4% DMSO.	No serum or serum products were used during growth and preservation.	Approx 1 hr	1 mon	8-9	Centrifuge, incubator, 2 l bottles with stoppers, bottle shaker, 60 ml bottles, Linde liq nitrogen refrigerator, ampules, serum bottles, refrigerator Nagles's medium (described), DMSO	Viable cell lines without serum or protein hydrolyzate contamination	Brown (1965)

FREEZING (Continued)

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158. Tissue culture cells	Cultured embryonic kidney tissue	Human embryonic kidney tissue was placed in NCTC 109 medium or Hank's balanced salt soln and refrigerated at 4°C until processed. The fibrous capsule was removed, and the kidney tissue was minced in Hank's soln. The tissue was then rinsed 3-6 times in a trypsinizing flask with Eagle's basal media in Hank's soln. Then the tissue was digested with enzymes, the collected suspension was centrifuged, the packed cells were resuspended, and finally the cells were planted in tissue culture media contg DMSO and frozen in sealed ampules to -196°C.	85% of all frozen kidney cell suspensions could be cultured after thawing. Cells from different kidneys required different times to produce confluent cultures, but cells from the same kidney tissue required the same time for confluency.	A continuous supply of human kidney cell cultures is needed for screening and production of viruses.	Approx 1 hr for freezing	At least 8 mon	> 10	Refrigerator, Petri dishes, surgical tools, trypsinizing flasks, Millipore filters, water bath, incubator, magnetic stirrer, funnel, gauze, centrifuge, bottles, ampules, screw cap tubes, ice bath, heat sealer, Linde BF 3-2 freezer, perforated plastic bags, Linde LNR 185 containers NCTC 109 soln, Hank's balanced salt soln, Eagle's basal medium, trypsin, Earle's saline soln, 2% calf serum, DMSO, liq nitrogen	Tissue culture bank	Perry (1965)
159. Tissue culture cells	Tissue obtained from an abortus	Tissue from 7 spontaneous human abortuses was minced, rinsed in Hank's balanced salt soln, then cut into pieces 2-4 mm in diameter. The tissue was placed in a medium of 80% lactalbumin hydrolysate with Hank's salt, 10% glycerol. The suspension was put in vials, sealed, and placed in cotton-filled containers for freezing to -80°C at 1-2°C/min. After several days the vials were thawed and the tissue was washed and recultured.	In all 7 cases cultures were successfully initiated after thawing. No adverse cytological findings were noted, and no segregant cells were observed.	The authors felt that the technique should also apply to preservation of adult tissue.	1-2 hr	Several days	6-7	Culture dishes, stoppered glass vials, cotton-filled paper cup, Revco freezer, water bath, tissue mincer Hank's balanced salt soln, 80% lactalbumin in Hank's salt soln, 10% fetal calf serum (Difco), 10% glycerol	Preservation of tissue and tissue cultures	Stanchever (1965)
160. Tissue culture cells	Previously cultured cells (7 yr old cell line)	The cryoprotective action of 26 chem agents on tissue culture prepn was investigated by testing the reproductive integrity of cells from a tissue culture cell line after freezing. The plating efficiency of individual cells was determined by Puck's cloning technique.	Cells frozen in normal tissue culture medium contg 0.5% lactalbumin hydrolysate and 5% calf serum in Hank's balanced salt soln had an av survival of 16.3%. The best cryopreservatives were: ethylene glycol, propylene glycol, glycerol, DMSO, pyridine-N-oxide, and hexamethylene tetramine. Others had lesser effect or were toxic to the cultures.		½ hr	Not given	> 10	Rubber stoppered bottles, ampules, alc baths, stirrer, Dewar flask, Petri dishes with plastic bottoms 0.5% lactalbumin hydrolysate in Hank's soln with 5% calf's serum, penicillin and streptomycin; dry ice; alc; liq air; trypsin	Evaluation of cryoprotective agents	Vos (1965)

FREEZING (Continued)

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161. Tissue culture cells	Tissue cultures	4 ml ampules contg human embryo fibroblasts, or one of five other cell lines, were control-rate frozen in 7.5% DMSO after heat sealing using a Linde BF 3-2 freezing app. Ampules contg each kind of cell were later removed from storage at liq nitrogen temp and stored for 9 days in cardboard cartons in a styrofoam box contg dry ice. On days 2, 4, 7, and 9 samples of each cell line were thawed and checked for viability.	Short term storage of tissue culture cells in dry ice for 9 days had no observable effects on cells previously stored for long periods in liq nitrogen.		< 1 hr	9 days at dry ice temp	3	Linde BF 3-2 freezer, styrofoam dry ice container, ampules, heat sealer, water bath 7.5% DMSO soln, dry ice, liq nitrogen	Shipment of tissue cultures in dry ice containers	Price (1968)
162. Tissue	Solid block of human tissue	Tissue blocks were frozen on the chuck of a rocking microtome using solid carbon dioxide. 5 μ tissue slices were cut at -20°C and collected in weighed pots. Sectioned tissue was eluted with normal saline buffered with phosphate to pH 7.6 (3 ml/gm sliced tissue). A slurry of tissue and buffer was agitated or mixed 3 hr and left standing overnight at 2°C. The suspension was centrifuged and the supernatant pipetted. Supernatants and/or unmacerated tissue sections can be stored at -20°C.	This technique was a superior method for homogenation, and it produced more disrupted cells than other standard methods.	The cells were not disrupted by sectioning, but by freeze-drying the slices, then bursting the cells by immersion in buffer.	1 day	Not given	6-7	Cambridge rocking microtome, containers for sample, pipettes, rotary mixer, deep freezer or Bright's cryostat Normal saline with phosphate buffer, dry ice	Tissue studies; anal of tissue proteins	Tee (1964)
163. Tooth dentin and cementum	Freshly extd human tooth roots	Human tooth roots were freed of soft org matter, cut into buccal and lingual halves 5-7mm long, with nicks made on both inner and outer root walls. Roots were placed in a freezer at -5°C immediately after extn for storage, then thawed and implanted into the subcutaneous tissue of rats for up to one yr. Host tissue reactions and histochemical changes in the transplanted tooth were noted.	Roots could be preserved up to 35 days in this manner with 27 days being the av. Freezing caused quantitative but not qualitative changes, and tissue reactions were seen earlier and to a greater degree than with fresh implants.		Not given	Up to 35 days	4-5	Surgical tools, freezer	Transplantation	Morris (1969)

FREEZING (Continued)

SPECIMEN DESCRIPTION	ORIGINAL FORM OF SAMPLE	BRIEF DESCRIPTION OF METHOD	LIMITATIONS OF METHOD	OTHER COMMENTS ABOUT METHOD	ESTIMATED PRESERVATION TIME	SURVIVAL TIME	NO. OF STEPS	EQUIPMENT AND REAGENTS USED	END USE OF SPECIMEN	REFERENCE
164. Toxoplasma gondii	Tissue culture suspension of infected cells and free organisms	The Rh strain of Toxoplasma gondii was cultured in vitro in 4 oz bottles on bovine kidney cells in Eagles minimum essential medium plus nonessential amino acids and 10% fetal calf serum. Infected cells were scraped from the culture bottle, transferred to centrifuge tubes, and spun for 30 min. The resulting pellet was resuspended in medium contg 7% DMSO, added to sterile ampules which were then sealed, and slowly frozen to -45°C. The ampules were stored in a liq nitrogen freezer.	Viable, infectious Toxoplasma were demonstrated by mouse inoculation after 333 days of storage.	Infected cell cultures incubated at 37°C did not survive longer than 7 days, but the cell culture method was still considered a good one for production of clean, uniform seed stock.	3-4 hr	333 days	9-10	4 oz bottles, rubber scraper, incubator, centrifuge tubes, centrifuge, ampules, heat sealer, refrigerator, liq nitrogen freezer, paper tubes, liq nitrogen freezer LR-25-G (Linde) 7% DMSO, modified Eagles minimum essential medium, fetal calf serum, mouse blood inoculum	Maintenance of type cultures	Paine (1969)
165. Trichomonas vaginalis	Pooled cultured organisms	Trichomonads were grown axenically on trypticase-yeast ext-maltose medium contg agar. After 72 hr cultures were pooled, centrifuged, and the flagellates resuspended in fresh medium without agar to which 5% DMSO was added. Samples were put in screw-top vials, frozen to -35°C at 1°C/min, then rapidly to -196°C, and finally were stored over liq nitrogen. Virulence of stored samples was assayed by a special mouse test.	Trichomonads thawed and subcultured after 2 yr showed no loss of virulence compared to nonfrozen controls.		1-1½ hr	2 yr	6-7	Culture dishes, centrifuge, screw-cap vials, Canalco freezing unit, liq nitrogen container Trypticase-yeast ext-maltose medium, agar, DMSO, liq nitrogen	Maintenance of microorganism line	Diamond (1965)

FREEZING (Continued)

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166. Triphosphopyridine nucleotide, reduced	Incubated mixt	TPN was prepared from sheep liver by ion exchange chromatography. TPNH was prepared enzymatically as follows: TPN was dissolved in 0.1 M phosphate buffer, pH 7.5. 0.1 M $MgCl_2$ and 0.05 M d-isocitrate were added. The reaction was started by a phosphate ext, pH 7.5, of washed dried pig heart contg isocitric dehydrogenase. The reaction was allowed to continue 30-40 min until optical density of 340 mμ was reached. The incubated mixt was adjusted to pH 9.0-9.5 and placed in a boiling water bath 3 min. The resulting suspension was centrifuged 5 min, and the supernatant was stored at -15°C as a TPNH source.	TPNH soln could be stored for months at -15°C and still maintain its extinction coefficient at 340 mμ.		Approx 2 hr	Several mon	5-6	Incubator, flasks, boiling water bath, centrifuge, freezer TPN; 0.1 M phosphate buffer, pH 7.5; 0.1 M $MgCl_2$; 0.05 M d-isocitrate; pig heart ext	Standard for enzyme studies	Nason (1953)
167. Trypanosomes	Heparinized blood contg live organisms	Blood samples contg *Trypanosoma brucei* were extd from infected live mice by cardiac puncture with heparin as anticoagulant. The blood mixt was cooled to 0°C, and 1 ml aliquots were added to Wasserman tubes contg glycerol or DMSO. Thick-walled capillary tubes were filled 2 cm with each sample and sealed. The capillary tubes were plunged into liq nitrogen at -196°C for approx 3 sec, then stored under liq nitrogen until used.	Samples stored without cryoprotectants dropped 1.3 log units after 4 days preservation. From 4-200 days, infectivity remained stable. Glycerol and DMSO did not protect the organisms against loss of viability or infectivity.	This was a very simple method that could be used for blood samples in general.	5-10 min	200 days at -196°C	4	Microhaematocrit capillary tubes, Dewar flask with liq nitrogen, hypodermic syringe, Wasserman tubes (75x10 mm), forceps, boxes Glycerol, DMSO	Maintenance of viable strains	Herbert (1968)
168. Trypanosomes	Cultured trypanosomes	*Trypanosoma brucei* stabilate TREU 370 was deep-frozen, thawed, and immediately examined by electron microscopy to see whether any cell abnormalities could be detected which would account for low infectivity immediately after thawing.	There is gross dilatation of the mitochondrial envelope of the DNA core of the kinetoplast.					Freezer	Infectivity studies with trypanosomes	Macadam (1970)

FREEZING (Continued)

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169. Tsetse flies or sandflies contg trypanosomes	Whole tsetse or phlebotomine sandflies	Whole, naturally-infected tsetse flies or phlebotomine sandflies were frozen 'dry' with no preservative or 'wet' in 8% glycerol and 10% DMSO made up in phosphate-buffered saline soln, fetal calf serum, or normal rat serum. Cooling rate was approx $2^{o}C/min$ down to $-40^{o}C$.	All 'wet' mixt resulted in good preservation of the whole insect. Live trypanosomes were recovered from the mouthparts, salivary glands, and midguts of the frozen flies after preservation up to 18 wk, even after having been thawed and refrozen. One infection of sandfly flagellate was cultured and successfully subpassaged.	Tissues were excellently preserved, and peristaltic movements were often seen in insects dissected after storage. The technique is useful for field work.	1-2 hr	> 18 wk	2	Liq nitrogen freezer 8% glycerol, 10% DMSO, phosphate-buffered saline soln, fetal calf serum, rat serum	Tissue culture of parasitic organisms living in the host insects, and histological studies of the host	Minter (1970)
170. Tumor tissue	Fresh tumor tissue (cells, strips, or pieces)	A variety of tumors (mostly mouse, rat, or hamster) were cultured, harvested, and placed in a freezing medium contg 75-80% Eagle's Basal Media with Hank's balanced salt soln, 15% horse serum, 10% DMSO or 5% glycerol, and 100 µg penicillin or streptomycin/ml. The samples were frozen in sealed ampules at $1^{o}C/min$ to $-60^{o}C$, then rapidly to $-190^{o}C$.	Viability and biol characteristics of most tumors tested may be preserved for long periods by use of frozen storage. Sensitivity of tumors to chem agents did not seem to be altered by this technique.	DMSO was a better cryoprotectant than glycerol for Ridgeway Sarcoma and murine chondrosarcoma.	2 hr	Up to 4 yr; av 1 yr	4-5	Linde BF-3-2 freezer or dry-ice freezer, strip recorder, thermocouple, ampules, Petri dishes, metal covers, stoppered vials Eagle's basic medium, Hank's balanced salt soln, horse serum, DMSO, glycerol, antibiotics	Tumor tissue bank	Kline (1964)
171. Tumor tissue	Freshly excised normal or neoplastic tissues	Dextran 3, 10, 20, 40, 80, and 120; PVP; glycerine; and DMSO were prepared with Hank's balanced salt soln in conc of 3, 5, 10, 15, 20, 25, 30, 35 and 40%. These soln were tested for protection of normal neoplastic tissues (such as hamster cheek pouch, Sarcoma 180, Novikoff hepatoma, mouse breast adenocarcinoma, and S91 Cloudman melanoma) against freeze-thaw damage to cellular nucleic acid- and glycine-related synthetic systems. Most soln were adjusted to pH 7.4 before use, and tissues were frozen at $1^{o}C/min$ from 4^{o} to $-35^{o}C$, then rapidly to $-70^{o}C$.	Glycerine and DMSO at 10 and 20% conc were greatly superior to other compounds tested. DMSO was effective in a broader range of conc than glycerine.		Approx 1 hr	Not given	5-6	Surgical tools, Petri dishes, Canalco freezer, storage freezer Hank's balanced salt soln, dextrans 3, 10, 20, 40, 80 and 120, PVP, glycerine, DMSO, sodium bicarbonate	Study of nucleic acid- and glycine-related synthetic systems in normal or neoplastic tissures.	Woolfrey (1964)

FREEZING (Continued)

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172. Tumor tissue	Freshly excised mouse tumor	Murine tumors were removed from donor animals at the optimal stage of growth and were placed in wide-mouthed glass vials contg 8 ml of a medium consisting of 75% Hank's balanced salt soln, 15% horse serum, with or without 20% glycerol or 10% DMSO. The tumors were agitated for 15 min on a shaker at $4^{o}C$, the medium was removed and the vials were flame sealed. Tumors were slowly frozen in a Linde BF-3-2 nitrogen freezer to $-60^{o}C$, then fast frozen to $-190^{o}C$ and stored at that temp.	Mouse tumors can be successfully stored by freezing in the presence of glycerol or DMSO. However, DMSO gives better protection. Percent of tumor takes on reimplantation was given for the various tumors studied, and enzyme activities after freezing were also given.		2 hr	Not given	6	Surgical tools, wide-mouth glass vials, mechanical shaker, flame sealer, Linde BF-3-2 freezer, thermocouple, Electronik 17 strip recorder, Linde LNR 640-B storage freezer Eagle's basal medium, Hank's balanced salt soln, horse serum, DMSO, glycerol, penicillin, streptomycin	Tumor preservation	Kline (1965)
173. Tumor tissue	Freshly excised tumor tissue	A large variety of neoplasms were diluted with a freezing medium contg 80% Eagle's basal medium, 15% calf serum, and 5% glycerol. They were then homogenized, and suspensions were sealed in ampules and frozen at $1^{o}C$/min to a final temp of $-70^{o}C$. Samples were stored in a liq nitrogen refrigerator. After storage they were inoculated into animals to test their viability.	53 out of 55 tumors remained viable up to 1 yr. 40-95% of ascites tumor cells survived storage without producing change in mean survival time when implanted in new hosts. Drug sensitivity of tumors was unchanged after freezing.		2 hr	1 yr	5-6	Homogenizer, ampules, liq nitrogen refrigerator, Canalco freezer 80% Eagles basal medium, 15% calf serum, 5% glycerol	Maintenance of tumor tissue bank	Wodinsky (1965)
174. Tumor tissue	Minced tumor tissue	Minced tumor tissue was suspended in Hank's balanced salt soln, 1:1, without addition of serum. Either glycerol, 5%, or dimethylsulphoxide, 7.5%, was added; and the mixt was placed in 4 ml quantities into plastic tubes and sealed. Samples were stored at $4^{o}C$ for 30 min then cooled in a freezer at $1^{o}C$/min to $-196^{o}C$.	40 tumor strains were tested and many showed 75-100% takes on transplantation after 1 yr. Others exhibited irregular behavior during storage. Hank's soln plus 5% glycerol or 7.5% DMSO was suitable for freezing most tumors tried. In some cases, however, a supercooling effect during freezing destroyed the tumor viability. This was overcome by hand-regulating the liq nitrogen influx during the critical time.		1-2 hr	$>$ 1 yr	6	Tissue mincer, plastic tubes, B-F-3-1 freezing app (Union Carbide Ltd), heat sealer, refrigerator, liq nitrogen freezer Hank's balanced salt soln, glycerol, DMSO	Tumor transplants	Gericke (1966)

FREEZING (Continued)

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175. Urine	Fresh canine urine or blood serum	Urine from a nephritic dog was placed in the barrel of a 20 ml plastic syringe and frozen at -6°C overnight. The plastic syringe barrel was placed on top of a 5 ml glass bottle in the bottom of a 100 ml centrifuge bucket, wrapped, and centrifuged for 1 min at 3,500 rpm at room temp. A yellowish liq was obtained from the glass bottle, leaving a cast of frozen colourless ice in the syringe barrel.	This technique will not work if the substance is frozen beyond a critical temp (not given).	This method was primarily a sepn method, but could be coupled with protein preservation. Water froze first in serum and urine, and other substances suspended or dissolved therein were trapped in the lattice work of ice crystals in a conc form, and were then removed by centrifugation. If all constituents are frozen the method will not work.	Few min	Not given	2-3	20 ml plastic syringe, 5 ml glass bottle, 100 ml centrifuge bucket, centrifuge wrapping material	Protein anal using Biuret method; sodium anal using Beckman spectrophotometer	McErlean (1966)
176. Urine	24 hr human urine specimens	Urine samples to be subjected to amino acid electrophoresis or chromatography were conc to a uniform level by first drying 2 ml aliquots down in vacuo over conc H_2SO_4. The dried samples were kept in a deep-freeze until needed, at which time they were reconstituted to 2 ml with 0.01 N HCl.	Urine samples handled this way may be kept for years. Asparagine and glutamine were especially stable in urine samples stored by this method.		Few hr	Several yr	3-4	Containers, dessicator, freezer H_2SO_4, 0.01 N HCl	Urine electrophoresis or chromatography	Pasieka (1968)
177. Urine	Freshly voided human urine	The effect of urine storage at room temp and at -20°C on ammonia content and pH was investigated using a direct method for ammonia detn.	Noninfected urine can be stored in sterile, stoppered bottles for 48 hr at room temp and for 8 wk at -20°C without change in ammonia content or pH.		Few min	48 hr at room temp; 8 wk at -20°C	1-2	Sterile test tubes, sterile stoppered bottles, paraffin sealer	Urine anal by Berthelot's indophenol reaction directly on diluted urine	Gips (1970)

FREEZING (Continued)

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178. Uterine horns	Freshly excised rat uterine horns	Female rats were killed after 6 days of diethylstilbesterol injections. The uterine horns were removed and incubated in a modified physiol saline soln contg varying amt of either glycerol or DMSO. The horns were finally plunged into liq nitrogen then thawed after 1 hr. The effects of cryoprotectant conc, incubation time, and temp on freezing and thawing damage were noted.	DMSO was no more effective than glycerol as cryoprotectant. Drug-induced contractions were normal, but electrolyte balance was altered with both substances. Slow thawing to reduce osmotic shock caused further electrolyte imbalance.	Sufficient time to allow intracellular penetration of the cryoprotectant seemed to be the most important step in preservation by this method.	Varied with method	Were thawed after 1 hr	Varied	Incubator, liq nitrogen container, air bubbler, freezer DMSO soln, glycerol soln, modified physiol saline soln, liq nitrogen, diethylstilbesterol	Study of cryoprotectants	Carroll (1968)
179. Uterine horns	Freshly excised rat uterine horns	Rat uterine horns were excised and incubated at 37°C in physiol saline soln contg MgSO4 or $MgSO_4$ and glycerol. The horns were frozen in liq nitrogen and thawed with physiol saline soln contg varying amt of calcium. The effects of these procedures on contractility were measured.	$MgSO_4$ alone or with glycerol was not an effective cryoprotectant for uterine horns frozen at -196°C. Both omission of, or a 10-fold increase in, calcium changed the electrolyte balance in thawed tissue for the worse.		Varied	Not given	3-4	Liq nitrogen freezer, incubator, containers Physiol saline soln, magnesium sulfate, glycerol, calcium	Freeze-thaw effect on uterine horn contractility	Lin (1968)
180. Viruses	Measles virus suspended in support medium	Attenuated measles virus was incubated 6-9 days at 32°C, pooled with maintenance medium, and centrifuged. Either DMSO or another protectant contg calcium lactobionate and normal human serum albumin was added, and samples were frozen at -76°, -40°, and -20°C. The samples were also lyophilized at 0°, -20°, -40° and -76°C. Comparisons of activity titres were made to determine optimum freezing and drying temp.	The smallest loss of titre (.19 log) was found in suspensions frozen at -76°C and dried at 0°C in Medium 199 plus Parke-Davis protectant. Samples frozen only, lost less activity than those both frozen and dried.	Lowest titres were found in suspensions stored for 180 days at -40°C. These were lower than those stored at -20° or -65°C.	Not given	180 days	3-4	Lyophilizer with special tube attachment, centrifuge, pyrex tubes, pipettes, water bath, ice bath or freezer DMSO, Parke-Davis Protectant (PDA) contg calcium lactobionate and serum albumin	Strain maintenance for research	Greiff (1964)

FREEZING (Concluded)

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181. Viruses	Cultured BVD virus strains	Cultures of BVD virus were given the following treatment prior to filtration. They were: 1) freshly grown in susceptible cells, 2) stored at -75°C for 1-4 yr, 3) frozen and thawed for 3 cycles of -75°C and 37°C, 4) sonicated at 20,000 cps for 10 min, 5) incubated with 0.1 mg/ml of ribonuclease for 30 min at 25°C. Samples were then centrifuged and ultracentrifuged, and the resultant changes reported.	Storage of cytopathogenic NADL virus for 1.5 yr at -75°C altered the viable particle size and increased the filterability, whereas storage of noncytopathogenic BVD virus for 3 yr at -75°C did not increase filterability significantly. Multiple freeze-thaw or sonication of the two types of viral cultures produced a reverse effect on filterability.	The authors decided that ultracentrifugation data did not provide sufficient information for calc of the sizes of BVD viruses. Sizes may be heterogenous and vary from strain to strain.	Not given	Up to 4 yr		Centrifuge, ultracentrifuge 0.1 mg/ml ribonuclease (others, if any, not given)	Viral culture maintenance	Fernelius (1968)

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REFRIGERATION

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182. Agar plates	Commercial or specially prepared fresh agar plates	126 cultures were tested on Mueller-Hinton agar plates which were stored for varying lengths of time at 4°C with or without plastic protective covers. Both commercial plates and prepared plates were used. The effects of plate age on the size of inhibition zones caused by applying Bauer-Kirby discs to the inoculated plates were noted.	Mueller-Hinton agar plates were stored at 4°C for 3 wk without an appreciable change in the size of inhibition zones. Storage of the plates in sealed plastic bags did not lengthen the shelf life of the plates noticeably.	Since the Bauer-Kirby method for screening antimicrobial drugs is becoming a practical clinical test, this work was done to find out how long agar plates used in the method could be stored without affecting the results.	Few min	Up to 3 wk	2-4	Plastic Petri dishes, refrigerator, polyethylene bags, commercial agar plates stored in Mylar bags (Hyland) Mueller-Hinton agar (Difco), 5% defibrinated sheep blood	Testing antimicrobial agents by the Bauer-Kirby method	Dewees (1970)
183. Blood	Freshly collected whole human blood	Blood from two human donors was expressed into siliconized flasks contg ACD. Aliquots were stored in capped containers at 4°C or placed in a 37°C water bath. All samples were swirled weekly. Analyses of nucleotide content were carried out on fresh samples and at intervals on stored samples until the samples were considered 'outdated'. The nucleotide metabolic breakdown pattern during storage was studied.	ATP followed the pattern ATP→ ADP → AMP → IMP→ hypoxanthine in ageing blood. ATP steadily decreased while hypoxanthine increased. The reactions took 8 wk at 4°C and 3 days at 37°C.	The reaction pattern of ATP breakdown to hypoxanthine might be useful to date storage blood or to compare different storage methods.	Few min	72 hr at 37°C, up to 8 wk at 4°C	3	Blood collection app, siliconized flasks, pipettes, refrigerator, capped bottles, water bath ACD soln	Transfusions	Bishop (1961)
184. Blood	Freshly drawn whole human blood	Whole blood samples for use in measuring blood pH should be stored in ice water, if the pH cannot be measured immediately after collection. Sodium fluoride can be used to delay glycolysis, but it does introduce some error.	Whole blood may be stored in ice water up to 2 hr with less than 0.015 units change in pH. For precise detn of PCO_2, blood samples should not be stored in ice water for more than ½ hr. Sodium fluoride between 10^{-2} and 10^{-4} M conc can be used to delay glycolysis; however, the conc is critical. Even when the conc was optimum, sodium fluoride introduced a variable error of 0.006 to -0.014 pH units.		Few min	½-2 hr	2	Blood collection app, containers, ice-water bath	Anal of pH and PCO_2 of whole blood	Gambino (1965)

REFRIGERATION (Continued)

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185. Blood	Freshly collected whole blood	Human blood was stored in its oxygenated form, and after deoxygenation with nitrogen, for 14-56 days at 4^oC in the following preservation media: ACD or CPD, adenine plus ACD or CPD, and adenine plus ACD or CPD buffered to pH 7.2 with tris(hydroxymethyl)aminomethane. Biochem changes during storage in these media were noted.	Storage of deoxygenated blood resulted in a rapid decrease of pH, an increase in lactic acid, and a less rapid change in oxygen affinities and 2,3-DPG levels. Storage at neutral pH maintained 2,3-DPG levels and oxygen affinity. Addition of adenine increased ATP somewhat. 2,3-DPG level was dependent on pH. Minor Hb levels were essentially unchanged.		$>$1 hr	Up to 56 days	Varied with method 4-7	Sterile plasma flasks, Swinnex disposable filter, bottle shaker, refrigerator Nitrogen gas, ACD soln, CPD soln, adenine, tris(hydroxymethyl)-aminomethane	Transfusions	Huisman (1969)
186. Blood	Freshly collected human blood	Freshly collected ACD-blood was left undisturbed at 4^oC for 12-24 hr. About half the supernatant plasma was removed under sterile conditions. The plasma was pooled and used for production of stable plasma fractions. The remaining unit of partly deplasmatized blood can be used for transfusions without further treatment.	Partly deplasmatized blood had hematocrit at near normal levels and may be stored for 3 wk at 4^oC. Its properties were essentially identical with whole blood.	This method was used in Switzerland and was proposed to cut down on collection of extra blood to produce plasma fractions.	12-24 hr	3 wk	4	Containers, blood collection app, refrigerator ACD soln	Transfusions	Bucher (1970)
187. Blood	Freshly drawn human donor blood	Blood was collected from young, non-smoking males in units (450 ml). During collection the blood was diverted into two Fenwal plastic bags each contg either ACD or CPD with or without added adenine and/or inosine preservatives. The bags were then stored at 4^oC in a blood bank refrigerator. Aliquots of these samples were used to determine oxygen dissociation curves and conc of 2,3-DPG. The changes in oxygen affinity which occurred during blood storage in these preservatives were summarized.	Deterioration of Hb in stored blood due to decline of 2,3-DPG conc in red blood cells occurred less in blood stored in CPD than in blood stored in ACD (probably due to the higher pH of the former). Adenine added to CPD-stored blood lowered 2,3-DPG, whereas inosine added to the CPD-adenine mixt, either initially or on day 25 of storage, regenerated 2,3-DPG so that Hb function could be preserved for a longer time during storage.	Inosine added at day 25 instead of initially prolonged storage an extra wk.	Few min	14-26 days optimum; up to 40 days sub-optimum	2	Blood bank collection app, Fenwal plastic bags, blood bank refrigerator Inosine, adenine, ACD and CPD anticoagulants	Transfusions	Dawson (1970)

REFRIGERATION (Continued)

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188. Blood	Freshly collected human blood	Various preservatives and additives for blood were investigated to see if high 2,3-DPG levels could be maintained in red blood cells during storage.	Inosine added to ACD-adenine blood on the 6th day (when DPG normally drops to half the normal value) kept DPG above 6th day levels for 8-12 days. Weekly additions of small amt of inosine to ACD-adenine blood maintained high levels of both ATP and DPG and also reduced toxic side effects of a large initial addition of inosine.		Not given	$2\frac{1}{2}$ wk ?	4-5	Blood collection app ACD, adenine, inosine	Transfusions	DeVerdier (1970)
189. Blood cells, Red	Freshly collected whole human blood	Whole blood was stored at 4°C in either ACD or CPD anticoagulant for 21 and 28 days. *In vivo* viability of the stored cells was measured, and it was found that the amt of ATP present was related to the postinfusion viability of stored cells. Correlation between poststorage ATP levels and red blood cell viability was not as significant.	ATP levels decreased less than 5% in the first 24 hr of storage at 4°C in ACD or CPD. After this time ATP began to decrease and the amt of decrease was related to the viability of the blood. CPD was the preferred anticoagulant for storage over 21 days, possibly due to the fact that it better preserves the ATP.	Other storage changes included a fall in plasma pH and a fall in the conc of plasma sodium, chloride, and whole blood dextrose. A rise was noted for plasma potassium, inorg phosphorus, and whole blood NH_4+. Mean corpuscular vol and osmotic fragility of red cells increased as did the conc of free plasma Hb.	Few min	21-28 days	3	Refrigerator, blood collection app, modified plastic blood containers, pipettes ACD anticoagulant, CPD anticoagulant	Transfusions	Dern (1967)
190. Blood cells, Red	Freshly drawn human blood	Rate of loss of 2,3-DPG was investigated under the following storage conditions: 1) storage in ACD soln, 2) storage in CPD soln with or without adenine at varying pH, 3) storage in CPD, 4) storage in ACD and CPD with or without adenine.	After 2 wk storage of blood at 4°C in ACD soln, 65-85% of the red-blood-cell 2,3-DPG was lost; slightly less in CPD. Adenine increased the rate of loss of 2,3-DPG, while an alk pH helped maintain 2,3-DPG levels but adversely affected ATP. 2,3-DPG is restored gradually on reinfusion, but the process may take more than a day.	Because of loss of 2,3-DPG, stored blood may fail to transport oxygen efficiently for many hr after reinfusion.	Few min	2-3 wk	Varied	Blood collecting app, Fenwal plastic bags, refrigerator, centrifuge, Dubnoff shaker, hypodermic syringes Citrate soln, glucose, adenine, phosphate buffer, CPD soln, ACD soln, heparinized plasma, penicillin, streptomycin, CO_2	Transfusions	Beutler (1969)

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191. Blood cells, Red	Freshly drawn human blood	Several units of blood were collected in ACD soln, or into ACD soln contg 0.5 mM adenine, and were stored in a blood bank refrigerator. Blood was separated into types A and O. Type O was reinfused as an autologous transfusion, or was given as a type-specific homologous transfusion, or was administered to type A recipients.	During storage plasma Hb and potassium increased - less with adenine, however. A small increase in osmotic fragility was noted on longer storage. Hematocrit and pH decreased. Adenine units had higher survival times and a 70% 24 hr posttransfusion survival even after 42 days of storage.	Eleven out of twelve recipients of type-specific homologous transfusions developed mild urticaria.	Few min	42 days	4	Blood collection app, refrigerator, flasks or bags ACD soln, 0.5 mM adenine	Transfusions	Shields (1969)
192. Blood cells, Red	Freshly collected human blood	The role of phosphorylated compds in red cell preservation was studied by incubating blood for 4 hr at 37°C with ACD and various combinations of adenine, adenosine, inosine, guanosine, and inorg phosphorus. Blood samples were then stored at 4°C. The effect of pH on many of the combinations was also investigated.	ACD plus adenine and inosine at pH 6.0 gave good storage for 3 wk. Almost all other combinations were good for 2 wk along with controls. Addition of adenine and inosine, adjustment of pH, and a short incubation period before storage increased ATP and DPG.		Incubated 4 hr	2-3 wk	Varied	Blood collection app, containers, refrigerator, incubator ACD-A preservative, inosine, adenine, adenosine, inorg phosphate, guanosine, CPD-A preservative	Transfusions	Chanutin (1970)
193. Blood cells, Red	Freshly collected human blood	Red blood cells from one donor were used to test the effect of colloids such as gelatin, high mol wt dextran, albumin, or hydroxyethyl starch as replacements for plasma in ACD-plasma preservative for red blood cells. Inorg phosphate or adenine added to the nonplasma mixt were also investigated as blood preservatives.	The ATP content was lower by 1/3 in all cases where plasma was replaced by another medium. Samples contg ACD plus adenine that were stored 4 wk showed 71.8% av survival 24 hr posttransfusion.	The authors also presented a detection method for impurities in adenine using thin-layer chromatography.	Not given	Up to 5 wk	Varied	Blood collection app, refrigerator, test tubes, centrifuge ACD, CPD, gelatin, high mol wt dextran, albumin, hydroxyethyl starch, inorg phosphate, adenine	Transfusions	Gänshirt (1970)

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194. Blood cells, Red	Freshly collected human blood	Units of blood collected into plastic bags contg ACD were stored overnight in a cold room and centrifuged in the morning. 0.9% NaCl soln was added to some samples before storage, inosine-adenine-guanosine soln was added to others, and the effects on K^+ and ATP were noted. CPD was compared with ACD as a packed-cell preservative. Some effects of ACD and temp on 2,3-DPG and ATP levels during storage were also investigated.	NaCl soln added to packed blood cells before storage altered K^+, ATP, lactate and Hb. Inosine-adenine-guanosine soln gave good results. Packed cells from ACD- or CPD-treated blood can be stored for 2 wk with as much success as whole blood. CPD was no better than ACD as a preservative for packed cells at 4°C for up to 3 wk, but may be better for longer storage times. Blood should be cooled to 15°C immediately after collection in order to prevent loss of 2,3-DPG, but some 2,3-DPG will be lost during the first wk of storage anyway.	Loss of ATP in packed cells stored in CPD was counteracted by a higher rate of glycolysis.	Few min	2-3 wk	3-5 depending on method	Blood collection app, bottles, triple collection bags, refrigerator, various packing materials, centrifuge, ice bath ACD, CPD, 0.9% NaCl soln, inosine-adenine-guanosine soln	Transfusions	Prins (1970)
195. Blood cells, Red	Freshly collected human blood	Blood samples were preserved with the following combinations of preservatives: ACD plus adenine; ACD plus adenine and guanosine; ACD plus inosine, adenine and guanosine. The results of these nucleosides on storage time and the metabolic state of the stored cells was reported.	ACD plus inosine, guanosine, and adenine prolonged red cell survival for a total of 6 wk or more. The metabolism of the red cells was improved also. However, uric acid was increased in serum recipients when more than 3 units of blood were transfused in short sequences. This tended to normalize in 24 hr.	A triple-bag storage system allowed tailored preservation, sepn, and recombination of the cell and plasma fractions.	Not given	5 wk	Not clear, 3-6	Blood collection app, triple plastic collection bags, refrigerator Adenine, guanosine, inosine, ACD	Transfusion	Seidl (1970)
196. Blood cells, Red	Freshly collected whole human blood	Blood from adult humans was collected into ACD-NIH-A anticoagulant in plastic bags. Other samples were collected in the same manner except that 0.25 and 2.50 µmoles/ml of adenine and inosine respectively were added. A fresh blood sample was drawn to detn the initial ATP level. The blood bags with additives were cooled in chipped ice for 30 min, then stored at 1°C for various periods of time. The ATP conc in red blood cells was measured after various lengths of storage time.	A 70% av survival of red-cell ATP occurred in blood stored up to 35 days with one addition of inosine and adenine, and up to 56 days with a double addition of the two amino acids.		30-45 min	Up to 56 days	4	Blood collection app, plastic storage bags, ice bath, refrigerator, pipettes Adenine, inosine, ACD-NIH-A anticoagulant	Transfusions	Strumia (1970)

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197. Blood cells, Red	Freshly drawn human blood	Studies were conducted on the effects of adenosine, adenine, inosine, and ouabain on ATP levels in stored blood. Also studied were the effects of trauma due to shipping or moving plastic containers of blood, and the possible interaction of the stored blood with the plastic container itself. All of the above studies were carried out on blood stored with various preservatives.	Phosphate enhanced preservation of ATP and normal oxygen dissociation, and in CPD soln viable and 'more physiologic' cells were maintained up to 4 wk. Adenine also preserved nucleotides and at 0.5 mM extended blood shelf life to 5 wk. An improved PVC plastic was the best plastic for storage. ACD, CPD and ACD-adenine gave approx the same yield of antihemophilic globulin cryoprecipitate indicating that any of them may be used.	Inosine may also extend red cell preservation time, but it had not been thoroughly investigated as of 1970. The storage temp was not altogether clear. It was assumed blood was refrigerated at $4^{o}C$ as usual.	Not given	4-5 wk	3-5; varied with method	Plastic containers, blood collection app ACD, CPD, adenosine, adenine, ouabain	Transfusions	Warner (1970)
198. Blood plasma	Freshly collected human blood	Non-esterified fatty acid conc in plasma stored at $4^{o}C$, $-20^{o}C$, and $20^{o}C$ for up to 17 days was detd by Novak's method.	Non-esterified fatty acid conc was unchanged after 17 days if stored at $4^{o}C$ or $-20^{o}C$. Heptane ext of plasma were stable for 7 days at $20^{o}C$.	This data did not agree with Forbes and Camlin (1959) probably because they used a less specific method and were titrating other org acids then those intended.	Few min	17 days	2-4?	Not given	Anal by method of Novak (1965)	Broechoven (1968)
199. Blood platelets	Freshly drawn human blood	Human blood was collected from fasting donors into a plastic bag contg ACD anticoagulant. The blood was centrifuged, and the upper 3/4 of the platelet-rich plasma delivered into a plastic transfer pack. Excess ACD was added to prevent clumping. The platelet-ACD mixt was centrifuged to sediment red blood cells, and the resulting platelet-rich plasma was finally ultracentrifuged to produce a platelet pellet. The pellet was washed, resuspended in wash fluid, and stored in plastic bags on ice until used. Glucose and fatty-acid oxidations were observed for various lengths of storage time.	Storage of human platelets at $4^{o}C$ for 24 hr reduced the oxidative capacity for glucose by 25% and for long-chain fatty-acids by almost 50%.	The degree of osmolarity of the medium was significant to the oxidation rates for oleic acid and glucose. Decreased osmolarity caused decreased oleic acid oxidation but increased glucose oxidation.	1 hr	Samples were stored up to 48 hr but not without damage	9-10	Blood collection app, centrifuge, plastic transfer pack, ice bath, plastic blood bags ACD anticoagulant soln, 300 mOsm potassium phosphate buffer	Transfusions	Cohen (1970)

REFRIGERATION (Continued)

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200. Blood serum	Freshly drawn human blood	The effect of refrigeration on serum potassium was investigated. Blood samples were analyzed for potassium after storage at room temp and at 4°C for 24 hr.	Refrigeration of clotted blood resulted in a large increase (up to 230%) in serum potassium, while storage at room temp resulted in only a slight increase.	Blood specimens for anal of serum potassium should be centrifuged as soon as possible, and specimens should not be stored in the refrigerator.	$< \frac{1}{2}$ hr	24 hr	2-4	Lustroid tubes, blood collection app, centrifuge, refrigerator	Anal of serum potassium by the flame photometer method	Goodman (1954)
201. Blood serum	Freshly drawn human blood	The effect of serum storage procedures on cephalin-cholesterol flocculation, zinc sulfate turbidity, phenol turbidity, and serum bilirubin were investigated. Samples of serum were stored at room temp or at 4°C with or without contact with the clot. Some samples were stored under mineral oil to keep them from reacting with air.	Decreased thymol and zinc sulfate turbidity values were observed in sepd serum that was refrigerated overnight. Phenol turbidity increased. Changes in thymol, zinc, and phenol turbidities were largely prevented by allowing serum to remain in contact with the clot during storage. Serum bilirubin conc decreased slightly during storage. Anaerobic storage prevented changes in thymol and zinc turbidities but not in phenol turbidity.		Few min	24 hr	2-4	Test tubes, refrigerator Mineral oil	Hepatic function tests	Yonan (1957)
202. Blood serum	Freshly drawn human blood	Blood from healthy adult East Africans was collected in dry universal containers, and the serum was separated within 1-2 hr after centrifuging. After sepn the blood serum was either stored at room temp or at 4°C. Electrolytes were estimated within 24 hr with a flame photometer.	At room temp potassium levels rose 7.5% after 2 hr. At 4°C, levels rose 23% in 2 hr.	Serum should be separated immediately for potassium anal. Anal for potassium should be carried out within 2 hr, and blood samples or serum should not be refrigerated.	Few min	Serum should be separated and analyzed within 2 hr	2	Centrifuge, tubes, refrigerator, blood collection app	Blood serum potassium anal using a flame photometer	Owor (1965)
203. Endolymph	Freshly extd guinea pig cochlear endolymph	Endolymph was taken from the third turn of a guinea pig cochlea with a small glass pipette and placed in a 30 µl vial. The collected fluid was refrigerated. Pure endolymph was obtained by a freezing technique' (lyophilization?).	Not given		Not given	Not given	Not clear; 4-6	Pipettes, 30 µl vials, refrigerator, lyophilizer (?)	Thin-layer chromatography of mucopolysaccharides	Ishiyama (1968)

REFRIGERATION (Continued)

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204. Epidermal cells	Cultured epidermal cells from sea lion or seal flippers	Epidermal samples from the flippers of seals and sea lions were minced and incubated at 33° and 37°C in a modified Eagle's minimum essential medium. Cells were pooled and suspended in fresh medium in tissue culture flasks. These flasks were incubated at several temp between 4°C and 37°C for at least 10 days (some up to 3 wk). Other cells were held up to 6 mon at 4°C, and then were tested for viability at 37°C.	Seal epidermal cells stopped growing at 17° to 12°C but were viable when returned to 37°C. Controls also stopped growing at 17° C. Sample cells held up to 6 mon at 4°C were viable when returned to 37°C indicating a high degree of adaptation to hypothermia. Control cells did not have this adaptability.	Pinnipeds offer unparalleled opportunities for investigation of cutaneous heat requirements and adaptation.	< 1 hr (after culturing)	6 mon at 4°C	3-4	Refrigerator, culture dishes, incubator, pipettes, shaker, flasks Eagle's minimum essential medium, Earle's balanced salt soln, fetal calf serum, sodium pyruvate, nonessential amino acids, L-glutamine, versene	Study of cutaneous cell cold adaptation	Feltz (1966)
205. Eye sclera	Whole scleral cup of cat eyes	Cat eyes were enucleated, washed with antibiotic, and refrigerated at 4°C up to 24 hr. The anterior segment was removed from the globe, the optic nerve was removed, and the eye was turned inside out and scraped to remove the retina, choroid, and pigment. After washing with antibiotic soln, the scleral cup was placed right-side out over a nylon mesh bag. It was then dehydrated in 95% anhydrous glycerine.	Preserved scleral grafts in cats were still intact after 110 days. Preserved grafts seemed to perform as well as fresh grafts. The grafts themselves were stored up to 12 wk before use.	A homograft reaction was rarely encountered in scleral grafts.	15-20 min	> 12 wk	4 for dehydration; 2 for refrigeration	Surgical tools, refrigerator, nylon mesh bag, beakers or containers Antibiotic soln (not identified), 95% anhydrous glycerine	Scleral grafts	Hassard (1967)
206. Gastric juice	Neutralized human gastric juice	Gastric juice was collected from human volunteers after intragastric neutralization with sodium bicarbonate, and the lactic dehydrogenase activity was measured immediately. Two samples were stored at 4°C and two at -20°C, and the lactic dehydrogenase activity was measured daily.	After 7 days, neutralized human gastric juice stored at 4°C had approx 88-94% of the initial lactic dehydrogenase activity. Specimens stored 7 days at -20°C had less than half of the activity remaining. Freshly collected specimens were preferred, but if storage cannot be avoided, 4°C was the best storage temp.		Few min	7 days	3-4	Collection app, pipettes, storage containers Sodium bicarbonate	Anal of enzymes in gastric juice	Fenton (1966)

REFRIGERATION (Continued)

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207. Haemocyanin	Haemocyanin prepd from Helix pomatia by the method of Heirwegh (1960)	Haemocyanin soln (80 gm/l in 0.1 M sodium acetate buffer, pH 5.7) was satd with carbon monoxide and stored in sealed tubes at 4°C in the dark. Each mon one tube was opened, the contents were satd with air, and diluted with half-satd disodium tetraborate, pH 9.2, to reduce light scattering. Absorbance for haemocyanin conc of 1 gm/l and a path length of 1 cm (K_{346}) was calc. Similar expt were carried out for haemocyanin stored in air.	A compd was formed between haemocyanin (from Helix pomatia) and carbon monoxide. One carbon monoxide replaced one oxygen molecule. However, 95% of the theoretical amt of carbon monoxide bound to haemocyanin could be recovered. The storage of haemocyanin under carbon monoxide prevented change in absorbance for up to 250 days. Upon reoxygenation the oxygen dissociation curve was sigmoidal.		½ hr	Up to 250 days	4-5	Tubes, tube sealer, refrigerator 0.1 M sodium acetate buffer, half-satd disodium tetraborate soln, pH 9.2	Study of haemocyanin properties	DeLey (1970)
208. Hearts and lungs	Canine whole heart and aortic arch; single lung with atrial cuff	Canine hearts and lungs were stored in vitro with hypothermia (2°-4°C) and hyperbaric oxygenation (3, 8 or 15 atm) for 24 or 48 hr. 3 atm gave optimal results for lung storage, while 8 atm was best for heart storage for 48 hr.	Transplanted hearts and lungs survived 1-10 days in vivo after preservation for 48 hr in vitro.	Recipients of lung transplants died of edema, while recipients of heart transplants died of tissue rejection.	20-30 min	Up to 48 hr	Differs with heart and lung; under 10	Organ preservation tank providing both hypothermia and hyperbaric oxygenation, surgical tools, perfusion cannula, subcutaneous cervical pouch Tis-U-Sol soln (Baxter Labs), sodium bicarbonate	Heart and lung transplants	Largiader (1965)
209. Heart	Freshly excised rat heart	The fate of rat heart heterotrophic transplants after prolonged storage under hyperbaric oxygen and hypothermia was investigated using two different decompression procedures for the stored organs.	All transplants which had been decompressed by an anaphrotropic method resumed contractions immediately after release of the aortic clamp and maintained a regular rhythm up to 4 days.	The heart rate of transplants varied from animal to animal. A diffuse necrotic process was observed in many grafts from animals killed 48 hr after transplantation, no matter what decompression method was used.	~1 hr	Up to 2½ hr	4-5	Perfusion app, pressure tank Ringer's soln, low mol wt dextran, tris-(hydroxymethyl)aminomethane, lignocaine, calcium heparinate, oxygen	Transplants	Bui-Mong-Hung (1968)
210. Heart	Whole excised rabbit heart	Rabbit hearts were excised, cannulated, and flushed with heparinized saline. The heart was then attached to a perfusion app and perfused at 4°C with Eagle's Minimum Essential Medium with 10% newborn calf serum and varying amt of electrolytes added.	Eagle's Minimum Essential Medium contg 0.2% glucose plus 143 mEq Na^+, 13-15 mEq K^+, and 9.8 mEq Ca^{++} per liter and equilibrated with 95% O_2-5% CO_2 could preserve rabbit hearts up to 48 hr.		< 1 hr	48 hr	5	Refrigerator, perfusion app, cannulas Modified Eagle's Minimum Essential Medium, heparinized saline	Heart transplants	Athreya (1969)

REFRIGERATION (Continued)

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211. Heart	Freshly excised dog hearts	Excised living dog hearts and cadaver hearts were stored at 4°C in Ringer's lactate soln with or without perfusion with various perfusates. The effect of hypothermia, perfusate, and hyperbaric oxygen on myocardial contractility was measured.	Preservation by perfusion with colloidal plasma expander (6% hydroxyethyl starch in 0.9% saline) at 4°C and normal pressure was the best method; however, all preservation methods produced a decrease in the contractile force.	Glutamic-oxalacetic transaminase was used as an indicator of myocardial contractility. More than 600 U/gm indicated lack of contractility, but lower levels did not always indicate viability.	Varied	24 hr	Varied	Surgical tools, refrigerator, perfusion app, thermistor probe, ECG app, polyethylene balloon, catheters, plastic bags, pressure transducer, oxygen bubbler, steel hyberbaric chamber Ringer's lactate soln, heparin, physiol saline soln, 6% hydroxyethyl starch, glucose	Heart transplants	Garzon (1969)
212. Humans, Schizophrenic	Lightly anesthetized, naked schizophrenic patients	Naked and slightly anesthetized schizophrenic patients were placed between rubberized blankets that contained rubber coils through which a refrigerant was circulated. The refrigerant entered at a temp of -2°C to -5°C during the induction period and lowered the skin temp by 20°C or more. Patients were treated up to 48 hr with this treatment and observations were reported.	In 17 instances the patient's temp was below 37°C but the metabolic rate was elevated, often more than doubled, during the induction stages. This was mostly due to shivering. Voluntary movements continued at body temp of 30°C and below. Flexor muscles contracted. Acetonuria was at times intense and carbohydrate depletion was suspected. Repiratory vol was large initially, but dropped later. Several blood changes were reported, and acidosis was prevalent. Respiratory regulation remained effective to a body temp of 25°C.			48 hr	2	Rubberized blanket with rubber coils contg refrigerant None except anesthesia	Study of human body function during hypothermia and treatment of schizophrenia	Dill (1941)

REFRIGERATION (Continued)

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213. Interferon	Fresh urine and serum from Sindbis virus-inoculated rabbits	Blood serum and urine from rabbits inoculated with Sindbis virus were immediately acidified with 2 M citric acid and dialysed at $4^{o}C$ at pH 2.1 with constant stirring against 40 or more vol of 0.1 M citric acid for 4-5 days with 4 changes in order to preserve and isolate interferon. Urine precipitate was removed by centrifugation and freeze-dried. The urinary interferon-like viral inhibitor was compared to the serum interferon to see if the two were similar in properties.	The urinary interferon-like viral inhibitor was stable at pH 2 for up to 5 days at $4^{o}C$. It was nondialysable but highly susceptible to tryptic action, and it was reasonably heat stable.		4-5 days	Not given	4-6	Visking dialysis tubing, stirrer, large beakers, refrigerator, centrifuge, lyophilizer 2 M citric acid, 0.1 M citric acid, phosphate-buffered saline	Detection of interferon by the biuret method using bovine serum albumin as a standard; also, anal by UV absorption at 280 mm	Bocci (1967)
214. **Kidneys**	Freshly excised canine kidney	Excised dog kidneys were stored under the following conditions: 1) hypothermia, 2) freezing at $-10^{o}C$, 3) freezing with hyperbaric oxygenation, 4) hyperbaric oxygenation only, 5) hypothermia and hyperbaric oxygenation, and 6) hypothermia, hyperbaric oxygenation, and perfusion combined. The last combination gave the best 24 hr preservation method.	100% of the kidneys perfused for 24 hr in the hypothermia, hypobaric oxygen chamber survived 2 wk after transplantation into animals in which immediate contralateral nephrectomy was performed.	pO_2 values of the perfusate were markedly raised, while pCO_2 values, initially low, showed a slight increase during the 24 hr preservation period. pH remained normal and constant.	20-30 min	24 hr or longer	4-5	Surgical tools, refrigerator, hyperbaric oxygen chamber, freezer, cannulation needles, pump, Tygon tubing, flasks, ivalon sponge, autoclave Perfusate contg low mol wt dextran in normal saline soln, 1% procaine, and heparin; oxygen; alc-formalin soln for sterilization	Kidney transplants	Ackermann (1966)
215. **Kidneys**	Freshly excised canine kidney	The effects of graded hypoxia at $37^{o}C$ and $7^{o}C$ on total tissue water were measured on <u>in vitro</u> slices of canine renal cortex with and without the presence of low mol wt dextran. Slices were 0.3 mm thick and weighed 25-30 mg. They were incubated 30 min in 3 ml of modified Krebs-Ringer-phosphate soln with constant shaking, while a gas-oxygen mixt was bubbled into the medium through a 22 gauge needle. After incubation, slices were blotted, weighed, dried at $105^{o}C$, cooled in a dessicator, and reweighed to determine tissue wt loss.	Cell vol was maintained by an active energy-requiring process of the cell. Hypothermia, hypoxia, or other metabolic deprivations increased the cell vol in tissue slices <u>in vitro</u>. Reduction of oxygen conc to 5% was required to produce significant cell swelling at $7^{o}C$. At 20% oxygen, or less, no significant hypothermic swelling occurred. Low mol wt dextran exerts a colloidal osmotic effect on <u>in vitro</u> tissues with reversal of hypothermic swelling.		Approx 1 hr	Not given	5-6	Surgical tools, microtome, incubator, shaker, 22-gauge hypodermic needle, balance, dessicator Low mol wt dextran, gas-oxygen mixt, modified Krebs-Ringer-phosphate soln	Study of cell vol changes during hypothermia and hypoxia	Enerson (1966)

REFRIGERATION (Continued)

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216. Kidneys	Freshly excised canine kidney	Dog kidneys, including the renal artery, vein, and ureter, were removed and perfused immediately with 10% dextran soln in physiol saline at 4°C for 5-8 min. When venous outflow was clear, the kidneys were placed in a sterile container contg Tis-U-Sol (Baxter Labs), and refrigerated for 24 hr at 4°C. Other samples were perfused at room temp and stored under oxygen pressure while a third set of samples were perfused at 4°C and stored under oxygen pressure and refrigeration. Results were compared.	Dogs survived more than 1 yr on transplanted kidneys stored 24 hr at 4°C under oxygen pressure.	Neither hypothermia nor hypobaric oxygen was as effective as the combination of the two for kidney preservation.	10-15 min	$>$24 hr	3	Refrigerator or ice bath, sterile containers, pressure chamber, surgical tools and sutures 10% low mol wt dextran soln, Tis-U-Sol electrolyte (Baxter Labs), oxygen	Kidney transplant	Ladaga (1966)
217. Kidneys	Whole fresh excised canine kidney	The right kidney of a normal adult dog was removed after a needle probe thermometer connected to a continuous recorder was inserted near the center of the kidney. The kidney was perfused with 100 ml of heparinized, autologous, arterial blood or Ringer's soln at a constant pressure of 1.5 meters of water using gravity drainage through the renal artery. The kidney was wrapped in lint and placed in a polythene bag contg 100 ml of TC 199 medium (Glaxo) at 4°C. It was then kept at 0°C in a hyperbaric chamber for various lengths of time after which the organ was reimplanted.	Kidneys preserved 24 hr after perfusion at 2-3 abs atm of oxygen with autologous arterial blood were capable of supporting life after delayed opposite nephrectomy.	The authors did not feel that hyperbaric oxygen contributed very much to the preservation of kidneys.	½ hr	24 hr	5-6	Hyperbaric storage chamber, surgical tools, needle probe thermometer, Kent recorder, perfusion app, lint wrapper, polythene bag TC 199 medium (Glaxo), cooled autologous arterial blood contg heparin, Ringer's soln	Transplants	Basso (1967)
218. Kidneys	Freshly excised canine kidneys	Dog kidneys were excised and perfused with a low mol wt dextran soln at 5°C in order to make the organs totally ischemic and cool. Then they were kept refrigerated at 5°C for various periods of time up to 96 hr. The kidneys were reimplanted and their function observed for periods up to one yr.	Reimplanted kidneys functioned for one yr after preservation of up to 20 hr by this method. Renal blood flow, PAH and inulin clearances, and intraarterial blood pressure were good up to one yr after reimplantation.		Approx 1 hr	Up to 20 hr	5-6	Surgical tools, refrigerator 1% procaine soln, 5% Rheomacrodex soln in saline, soln contg eq parts 10% invert sugar and 1.3% bicarbonate	Kidney transplants	Brunius (1967)

REFRIGERATION (Continued)

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219. Kidneys	Whole, freshly excised canine kidneys	Canine kidneys were preserved for 24 hr by hypothermia, hyperbaric oxygen, and continuous low pressure perfusion with both sanguineous and nonsanguineous media (described).	Canine kidneys were successfully reimplanted after storage at low temp and pressure for 24 hr in a blood-heparin-TC 199 medium.		Few min	24 hr	4-5	Vickers' hyperbaric oxygen perfusion unit with attached refrigeration unit (described), bottles, cannulas 3 different storage media (50% homologous blood in TC 199 with 5000 units of heparin was most successful)	Kidney transplants	Hendry (1968)
220. Kidneys	Whole or sliced rat kidneys	Whole or sliced rat kidney was placed in flasks with 5 ml of Krebs-Ringer-bicarbonate buffer without glucose; gassed with oxygen-carbon dioxide, 95:5 (v/v); stoppered; and stored at 4°C without shaking. Sterile precautions were not taken, but some samples had 100 mg streptomycin and 100 units of penicillin added.	Amino acid transport in the preserved samples was similar to that of fresh tissue for up to 24 hr. Activity dropped to less than half after 8 days of storage.		5 min	24 hr	3	Flasks, refrigerator Buffer, oxygen, carbon dioxide	Study of amino acid transport by kidneys	Lowenstein (1968)
221. Kidneys	Freshly excised dog kidney	Excised dog kidneys were immediately flushed with 5% Rheomacrodex, preceded in some cases with 0.5% procaine soln contg heparin until the perfusate was clear. Then a soln of eq parts 10% invert sugar and 1.3% bicarbonate at 5°C was infused into the kidneys. The organs were wrapped in gauze moistened in perfusate and stored at 5°C. After varying lengths of storage, the kidneys were reimplanted.	Organs preserved up to 20 hr with this technique can be reimplanted successfully. Function studies showed that some damage occurs to tubules and glomeruli, but that autologous implanted kidneys can survive successfully a year or longer.	The authors felt that a prolonged cool ischemia period induced immediately after kidney removal was essential, as warm ischemia is highly deleterious.	<1 hr	20 hr	5-6	Surgical tools, catheters, gauze compresses, refrigerator 5% soln of Rheomacrodex in normal saline, 1% procaine soln, 10% invert sugar soln buffered with bicarbonate	Kidney transplants	Brunius (1969)

REFRIGERATION (Continued)

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222. Kidneys	Heparinized whole rabbit kidney *in situ*	Kidneys were bathed in 10 mg of papavarine and preserved *in situ* in rabbits after the animal had been given 5-10 mg of heparin. The kidney and clamped pedicle were stored in the finger of a surgical glove and surrounded by iced saline slush at -0.6°C in the rest of the glove. The glove was inserted into a cooling coil and maintained at 0°C in stirred saline. At the end of storage, clamps were removed, the kidney was excised, and serum creatinine values were detd for 7 days. This method simulated removal and reimplantation without the variability.	The kidney could be stored up to 12 hr without perfusion. Creatinine values were 1.9 mg% for 8 hr and 2.6 mg% for 12 hr storage.	Simple heparinization prevented clotting for at least 12 hr of storage. ½ hr of warm ischemia following 7½ hr of hypothermic storage was damaging, and might degrade the results of storage; therefore, a cooling device should be used during implantation.	13 min from clamping the artery to cooling to 0°C	8-12 hr without perfusion	6	Rubber glove or suitable container, cooling bath, bulldog clamps Saline soln, heparin, papavarine	Kidney transplants	Collins (1969)
223. Kidneys	Freshly excised dog kidneys	Dog kidneys were excised and flushed free of blood with 4°-8°C heparinized Ringer's lactate soln. The renal artery was cannulated, and the kidney placed in chipped ice. Slow perfusion was begun from a height of 1 m with ice cold saline, saline with 6% clinical dextran, or saline plus 6 gm/100 ml conc human albumin. Angiograms, wt changes, and gross changes were noted after various lengths of storage as indicators of degree of vascular bed obstruction.	Perfusion with albumin soln gave a wt gain of less than 2%, a good angiograph pattern, and a flow rate of 14 ml/min after 3½ hr. Dextran perfusion was next best, while saline perfusion was least effective.		Few min	3½ hr	5	Surgical tools, cannulas, ice bath, perfusion app Heparinized Ringer's lactate soln, cold saline, saline with 6% clinical dextran, saline with 6 gm/100 ml conc human albumin	Kidney transplants	Dienst (1969)
224. Kidneys	Whole excised, baboon kidney	ATP was measured in the renal artery, vein, and urine of 33 baboon kidneys during isolated bloodless perfusion, under normothermic, normobaric conditions and oxygen or helium exposure in the fresh state, or after 24 hr hypothermic (4°C), hyperbaric (3 atm) preservation. Change in ATP level indicated a change in kidney viability.	Helium-exposed kidneys did not utilize ATP as well as oxygen-exposed ones. ATP had a direct renal vascular effect on kidneys perfused under either gas with evidence of some renal ATP utilization. ATP levels after 24 hr indicated depressed cellular enzyme function and mitochondrial O_2 uptake capacity.	The authors suggested that kidney preservation under helium should be further investigated.	Not given; <1 hr	24 hr	6-7	Pulsatile perfusion system, surgical tools, refrigerator, chamber with temp and pressure control, containers Heparinized saline, soln of 5% invert sugar and 6% dextran in saline, 8% $NaHCO_3$ aq soln, urea, oxygen gas, helium gas, ATP in isotonic saline	Kidney transplants	Murphy (1969)

REFRIGERATION (Continued)

SPECIMEN DESCRIPTION	ORIGINAL FORM OF SAMPLE	BRIEF DESCRIPTION OF METHOD	LIMITATIONS OF METHOD	OTHER COMMENTS ABOUT METHOD	ESTIMATED PRESERVATION TIME	SURVIVAL TIME	NO. OF STEPS	EQUIPMENT AND REAGENTS USED	END USE OF SPECIMEN	REFERENCE
225. Kidneys	Freshly excised dog kidney	Dog kidneys were perfused at normal atm pressure with modified, undiluted, microfiltered plasma for up to 24 hr at 8°-10°C. The system was completely closed, and the pulsatile flow was approx 1 ml/gm/min. The effect of the perfusion technique on metabolism and hydrogen ion activity was investigated.	Kidneys gained up to 13% in wt over 24 hr preservation, but tissue damage was minimal. All animals survived autografts with preserved organs for 30 days. Oxygen consumption, surface pH, lactate/pyruvate ratio, and perfusate potassium were relatively normal.		< 1 hr	24 hr	4-5	Perfusion app including silastic membrane oxygenator, pulsatile pump, refrigerator unit, heat exchanger; silastic tubing; Fenwal blood packs; transfer packs; cannulas; electrometer; glass electrode; anaeroid manometer Neomycin, plasma perfusate (modified Belzer), Ringer's soln, Solucortef (Upjohn), heparin, oxygen	Kidney transplants	Alexander (1970)
226. Kidneys	Whole excised dog kidney	Canine kidneys were stored in physiol saline with ice slush at 0°C for 16-30 hr using surface cooling alone or combined with initial perfusion with various soln. A special perfusate (described) plus infusion of mannitol and phenoxybenzamine gave the best results.	Perfused kidneys were viable for 24-30 hr. The av max creatinine was 1.9 mg/100 ml.	Use of the special perfusate gave consistent improvement over simple hypothermia. The effect of warm ischemia on the organ before preservation was not considered; therefore, the method may not be useful for cadavers.	Few min	24-30 hr	4-5	Styrofoam box, polypropylene bottle, surgical tools, oxygen bubbler, hypodermic syringes Perfusion soln, mannitol, pentobarbitone, phenoxybenzamine, pentobarbitone	Kidney transplants	Collins (1970)
227. Kidneys	Fresh intact dog kidney	An exptl model was presented for comparing warm ishcemic dog kidneys with perfused ischemic kidneys without transplantation. Ringer's soln with or without heparin and xylocaine, dextran soln, diluted dog blood, dog plasma, and other perfusates were tested at various temp for their effectiveness in renal preservation.	Perfused kidneys were damaged by perfusion and functioned more poorly than warm ischemic kidneys. Rapid cooling and gentle flushing with a small vol of perfusate contg heparin and xylocaine gave the best results. No particular perfusate was preferred.		15 min	2 hr	4-5	Surgical tools, bulldog clamp, Rochester needle, tubing, perfusate equipment Heparin, dextran, xylocaine, Ringer's soln, dog plasma, dog blood	Preservation of kidneys for transplants	Hardner (1970)

REFRIGERATION (Continued)

SPECIMEN DESCRIPTION	ORIGINAL FORM OF SAMPLE	BRIEF DESCRIPTION OF METHOD	LIMITATIONS OF METHOD	OTHER COMMENTS ABOUT METHOD	ESTIMATED PRESERVATION TIME	SURVIVAL TIME	NO. OF STEPS	EQUIPMENT AND REAGENTS USED	END USE OF SPECIMEN	REFERENCE
228. Kidney and ileum	Freshly excised canine kidney and ileum sections	Canine kidneys and ileal sections were removed and stored by one of four methods: 1) hypothermia at 2°C and hyperbaric oxygen pressure of 3 atm, 2) hypothermia and hyperbaric pressure of 15 atm, 3) supercooling to -5°C and hyperbaric oxygen of 3 atm, 4) intermittent perfusion, hypothermia, and hyperbaric oxygen pressure of 3 atm. All organs were perfused with low mol wt dextran soln contg 10% DMSO. Results of storage by the four methods were compared.	Renal function sufficient to sustain life 1 yr will return to a reimplanted canine kidney stored 24 hr at 2°C under 3 atm hyperbaric oxygen. Ileal sections stored the same way for the same length of time remain viable and absorb glucose 1 yr after cervical reimplantation. Higher pressures of 8-15 atm were detrimental to renal function.	All dogs living to 1 yr with reimplanted preserved kidneys were hypertensive.	Varied	24 hr	Varied	Refrigerator, surgical tools, pressure chamber, stainless steel basket 5% low mol wt dextran soln in saline, heparin, tris(hydroxymethyl)aminomethane buffer (THAM), 10% DMSO	Transplants	Rudolf (1967)
229. Killifish	Whole live killifish	Adult killifish were acclimated in a controlled temp salt water aquarium to 20°C for 22 wk, and then to 10°C for 32 wk, and finally were placed in water at -1.5°C for varying times up to 63 days. The fish were injected with either saline or glucose, once at 10°C, then daily at -1.5°C until they died. The effect of these injections on survival at subzero temp was reported. Glucose was also added directly to the water and its effect observed.	A hyperglycemic response was observed at once when fish were transferred to -1.5°C, and the increase in serum glucose was accompanied by depletion of hepatic glycogen. When serum glucose levels fell to normal, the fish began to die. Glucose added to the water significantly increased cold tolerance, while injected glucose did not.	Liver proteins were unaffected by temp from 20° to -1.5°C, while muscle proteins decreased slightly after long exposure to cold.	52 wk were needed for adaptation to -1.5°C	3 out of 14 were alive after 84 days at -1.5°C in glucose-treated water		Salt water aquarium with temp control, hypodermic syringes Saline soln, glucose soln	Study of cold adaptation in fish	Umminger (1970)
230. Knee joints	Whole canine knee joint	Knee joints were removed from dogs killed with sodium pentothal and were preserved in paraffin oil at 4°C. Before use the specimen was washed and left for 1 hr in physiol saline contg antibiotics.	Specimens were kept for 2 wk in paraffin oil at 4°C.	Knee joint grafts with preserved specimens lasted 4-8 wk then began deteriorating due to the formation of cysts filled with fibrous tissue. There was no discussion as to whether the method of preservation was a contributory factor.	Few min	2 wk	3	Surgical tools, containers, refrigerator Paraffin oil, sodium pentothal, physiol saline soln, antibiotics (not specified)	Knee joint transplants	Fiala (1968)

REFRIGERATION (Continued)

SPECIMEN DESCRIPTION	ORIGINAL FORM OF SAMPLE	BRIEF DESCRIPTION OF METHOD	LIMITATIONS OF METHOD	OTHER COMMENTS ABOUT METHOD	ESTIMATED PRESERVATION TIME	SURVIVAL TIME	NO. OF STEPS	EQUIPMENT AND REAGENTS USED	END USE OF SPECIMEN	REFERENCE
231. Liver	Freshly excised puppy and adult canine livers	Livers of puppies and adult dogs were perfused with Ringer's lactate soln contg penicillin and sodium bicarbonate at $4^{o}C$; then they were excised. The livers were injected with heparin and perfused with balanced salt soln contg insulin and pencillin. The organs were lowered into a hyperbaric perfusion chamber and perfused under 3 atm of oxygen. Livers were reimplanted and the effects of preservation were observed.	Puppy livers can be preserved 24 hr under pulsatile perfusion with a balanced salt soln at 3 atm of O_2; however, function was altered, and it was not demonstrated that reimplantation of perfused livers could sustain life for a prolonged period of time.		½ hr	24 hr	6-7	Surgical tools, Bird respirator, hypodermic syringes, cannulas, hyperbaric perfusion app. (described) Ringer's lactate soln, penicillin, sodium bicarbonate, balanced salt soln, oxygen	Liver transplants	Slapak (1967)
232. Liver	Freshly excised dog liver	Orthotopic canine liver transplantation was performed using various techniques with homografts preserved 8-25 hr after removal from the donor. The most effective technique involved hypothermia at $4^{o}C$, hyperbaric oxygenation at 3½-4 atm, and divided perfusion with diluted homologous blood at a total rate of 6 ml/gm tissue/hr. All dogs were treated postoperatively with immunosuppressants.	Livers preserved 8 hr gave similar transplant results to controls. Only 1 dog died in 19 days, and one was alive after 4 mon. Livers preserved 24 hr and transplanted, caused death in all recipients within a few hr or days from ischemic necrosis.		Varied with method	8-24 hr	Varied with method	Ice bath, cannulas, hyperbaric chamber, oxygenator Balanced electrolyte soln contg dextran, glucose, magnesium sulfate, and procaine; whole blood; 5% lactated Ringer's soln; ϵ-aminocaproic acid; protamine sulfate; heparin; azathioprine; prednisone; antilymphocyte globulin	Liver transplants	Brett-schneider (1968)
233. Liver	Freshly excised porcine liver	Excised porcine livers were perfused with cold ($10^{o}C$) heparinized, homologous, oxygenated blood diluted to a hematocrit of 15% with 50% lactate Ringer's soln in 50% dextran soln. Portal vein flow was 150 ml/min, and hepatic artery flow was 40 ml/min. Livers were preserved up to 24 hr in this manner and were then transplanted into recipients. Some recipients received ϵ-amino caproic acid, but none received immunosuppressive drugs.	Transplants that had been preserved up to 8 hr survived and functioned normally, except for serum transaminase. After 3 wk signs of rejection were noted. Recipients of organs preserved 18-24 hr died within 5 hr of transplantation.		<1 hr	8 hr	3-4	Perfusion app, surgical tools 50% lactate Ringer's soln in 50% dextran soln; heparinized, homologous, oxygenated blood; ϵ-amino caproic acid	Transplants	Mieny (1968)

REFRIGERATION (Continued)

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234. Liver	Freshly excised porcine liver	Pigs were heparinized, bled to death, and their livers removed. The livers were flushed with Ringer's lactate soln contg procaine and bicarbonate, then attached to a perfusion app where they were perfused at 8°-10°C with a mixt of porcine plasma (which had been collected in ACD soln and cryoprecipitated to remove labile lipoproteins), magnesium, cortisone, penicillin, and dextrose. After perfusion from 8-24 hr the livers were reimplanted, and the effects of the prior preservation were noted.	Porcine livers perfused with cryoprecipitated plasma could be preserved effectively up to 10 hr. Organs preserved for 24 hr and reimplanted developed bleeding diathesis.	Animals receiving transplants of preserved livers lived from 5-34 days and most died of tissue rejection.	< 1 hr	Up to 10 hr	6-7	Surgical tools, purfusate app (described), plastic bags, cannulas, micropore filters Heparin, Ringer's lactate soln contg procaine and bicarbonate, cryoprecipitated porcine plasma, ACD soln, magnesium, cortisone, penicillin, dextrose	Liver transplants	Belzer (1970)
235. *Neurospora crassa*	*Neurospora crassa* cultures grown on Fries medium	Cell-free ext were prepared from mats of wild *Neurospora crassa* grown on Fries basal medium. Mats were collected on a Buchner funnel, triply washed with distd water, and frozen 1-3 hr at -15°C. They were then homogenized in 3x their wt of cold 0.1 M K_2HPO_4 and centrifuged at 4°C. The cell-free, turbid supernatant was used for studies of the nitrate reductase content of mycelia and for further enzyme purification.	85% or more of the nitrate reductase activity of the homogenates was present in ext prepared in this manner.		> 1 hr (not counting culturing)	Not given; samples were frozen from 1-3 hr during the procedure	5-6	Buchner funnels, glass homogenizer, centrifuge, bottles, flasks Fries basal medium, distd water, 0.1 M K_2HPO_4	Further studies of cell-free enzymes in *Neurospora crassa*; nitrate reductase purification	Nason (1953)
236. Oviductal fluid	Fresh oviductal fluid from live monkeys and New Zealand rabbits	A system for continuous collection of oviductal fluid from monkeys was described. The uterotubal juncture was ligated, and the cannula fixed at the fimbriated extremity and brought through a stab wound in the lateral abdominal wall. Externally the extremity was connected to a special insulating chamber to which refrigerant was delivered. Accumulated fluid was maintained at 3°-5°C. The monkeys were restrained, and superovulation was induced with human menopausal gonadotropin and human chorionic gonadotropin. The method was also applied to New Zealand rabbits.	3 out of 13 monkeys yielded fluid suitable for assay. Most of the other monkeys died from stress or strangulation or excreted blood into the tubal fluid making it unfit for anal. Rabbits yielded fluid continuously for 2 mon. Many artifacts affected the continuous collection of oviduct fluid, but the method was useful for studying changes occurring during the menstrual cycle.	The method allowed a daily assessment of tubal fluid throughout the menstrual period.	Approx 1 hr	Up to 2 mon	5-6	Specially designed oviductal fluid collection system (described), surgical tools, cannulas, sutures, adhesive tape, restraining chair, hypodermic syringes Human menopausal gonadotropin and chorionic gonadotropin	Study of Fallopian tube fluid composition throughout the menstrual cycle	Mastroianni, Jr (1969)

REFRIGERATION (Continued)

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237. Phosphatase substrate	Freshly prepared substrate	Sodium glycerophosphate, 2.5 gm, and monosodium diethylbarbiturate, 2.12 gm, were dissolved in water in a 500 cc volumetric flask. Aliquots of 100-250 cc were stored in the refrigerator in glass stoppered bottles under a 3 cm layer of washed petroleum ether (b.p. 30°-36°C).	When the substrate was covered with petroleum ether and stored up to 2 mon in small bottles in the refrigerator, the activity ratios were 1.0 $\pm$ 0.03. Absorption of carbon dioxide by alk substrates could cause a change in pH.		Few min	> 2 mon	3-4	Volumetric flasks, glass-stoppered bottles, refrigerator, washed petroleum ether Sodium glycerophosphate, monosodium diethylbarbiturate, petroleum ether	Anal of serum phosphatase activity	Bodansky (1933)
238. Rats	Live rats	Long-Evans rats were cooled by placing them in plexiglas cylinders with an open top and a floor of spaced plexiglas rods. The chambers were put in a constant temp room, and the body temp of the animals was measured with a thermocouple inserted rectally and taped to the tail. Rats were injected i.p. with varying amt of DMSO in physiol saline soln. Some of the rats were shaved. The animals were maintained in 1°, 15°, and 25°C environments, and the effects on body temp were noted.	Shaved rats receiving 3 gm/kg of DMSO in a 1°C environment cooled to a 10°C body temp in 239 min. Animals receiving 6 gm/kg DMSO cooled to 10°C in 175 min. Shaved controls required 412 min to reach 10°C. Unshaven rats in a 1°C environment receiving 6 gm/kg DMSO cooled to 10°C in 383 min, while unshaven controls failed to become hypothermic. Shaved animals receiving 6 gm/kg DMSO at 15°C gave a mixed response. At 25°C, DMSO-treated animals dropped their temp 3.6°C and then rewarmed, while controls remained normothermic.	DMSO-treated animals cooled 2.5-3.9 times as fast as controls.	175-412 min	Several days at 1°C unshaven	6-8	Plexiglas cylindrical cages, constant temp room, copper constantin thermocouples, tape, razor Ether, DMSO, physiol saline soln	Study of DMSO-induced hypothermia in whole animals	Panuska (1966)
239. Soil	Fresh topsoil from under grazed pastures of grasses and clovers	The effects of 4 different methods of storage on the dehydrogenase activities of topsoils were detd. Samples were sieved, thoroughly mixed, and stored in the dark in separate samples in polythene bags. The samples were then stored by one of the following methods: 1) air dried 48 hr at room temp, then stored at 20°C, 2) undried and stored at 20°C, 3) undried and stored at 4°C, and 4) undried and stored at -20°C. Dehydrogenase activity was measured before and after storage and the results compared.	Storage at 4°C or -20°C was the most satisfactory for retaining dehydrogenase activity. A slight increase in dehydrogenase activity was noted after 77 days when compared to undried samples stored overnight at room temp. Drying and storage at 20°C was unsatisfactory as half the dehydrogenase activity was lost. Some topsoils could be stored undried at room temp for less than 77 days, but refrigeration or freezing gave better results.		Few min to 2 days	Up to 77 days	5-7	Containers, 2 mm sieve, polythene bags, rubber bands, refrigerator, freezer	Dehydrogenase detn	Ross (1970)

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240. Spermatozoa	Fresh ram ejaculate	High motility ram ejaculates were diluted 40-50 fold with diluents contg the following: 1) lactose plus varying ratios of potassium to sodium and magnesium to calcium, 2) varying amt of potassium, calcium, and magnesium plus varying amt of casein, 3) fructose, glucose, lactose, or sucrose plus varying amt of sodium chloride, 4) lactose and skim milk. Motility of the sperm were scored after incubation at 37°C, slow cooling, and storage at 5°C.	Sperm were viable up to 5 days at 5°C depending on the diluent.	The storage temp influenced the effectiveness and optimum amt of diluent needed.	Few min-2 hr	Up to 5 days	3	Refrigerator, glass tubes, diluents, pipettes A large no. of reagents were tried including sugars, electrolytes, proteins, and antibiotics	Artificial insemination	Martin (1966)
241. Spermatozoa	Fresh bull ejaculate	Bull semen was extended with a variety of extenders including Cornell Univ Extenders CUE and CU-16, and modified Illinois Variable Temp Extender. Other substances such as catalase, sodium bicarbonate, and sodium citrate were also added in varying amt as buffers or modifiers. All buffers had 15-20% egg yolk (by vol) added. Air, nitrogen, or carbon dioxide was used in the ampules above the semen mixt before sealing. All of these factors were compared to determine the best preservation method for unfrozen bull spermatozoa.	Up to 42% of the spermatozoa were motile 30 days after storage at 5°C. The best technique included extending with CUE with added catalase under air.	The method is useful when freezing app is not available.	½ hr	30 days	Varied with expt	Containers, glass ampules, plastic tubes, incubator, vacuum sealer, heat sealer Cornell Univ Extenders CUE and CU-16, Illinois Variable Temp Extender, penicillin, streptomycin, carbon dioxide, nitrogen, air, catalase, sodium bicarbonate, sodium citrate, egg yolk, Dow Antifoam C	Artificial insemination	Foote (1967)
242. Spermatozoa	Fresh bull ejaculate	DNA was determined in bovine spermatozoa stored in Cornell Univ Extender at 5°C in sealed ampules. The samples were exposed to light, dark, air, nitrogen gassing, and addition of cysteamine to determine the effect of these treatments on DNA and sperm nuclear size.	Light decreased the sperm motility and increased the nuclear area. Light also reduced DNA from 4.52 to 3.70 relative units. In nitrogen many nuclei were shrunken, as were nuclei of sperm stored in the dark with air or nitrogen. Cysteamine was toxic to spermatozoa and caused complete disappearance of nuclei exposed to light in an air atmosphere.			Up to 12 days		Glass ampules, sealer, refrigerator, light source Cornell Univ Extender, air, nitrogen gas, 0.5 M β-mercaptoethylamine (cysteamine)	Anal of DNA by Feulgen-positive staining and microspectrophotometry	Paufler (1967)

REFRIGERATION (Continued)

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243. Spermatozoa	Fresh turkey semen	Pooled samples of semen were collected by digital massage from 2 strains of turkey and were aspirated into 10 ml centrifuge tubes held in vacuum flasks at 16°C. These tubes were removed to a beaker of water at 16°C and cooled slowly (7.5°C/hr) to 4°C. The semen was diluted with a special diluent contg several sugars, sugar alc, and antibiotics. The effect of stored semen on fertile egg production and poults was noted.	Hens laid about the same no. of eggs; however an initial high fertility in the hens was followed by a general decline. Approx 75% fertility in semen was noted after 3 wk.	There was a high hen mortality in hens bred with preserved diluted semen. Bronze turkeys performed better than white turkeys.	2-3 hr	3 wk	4	Centrifuge tubes, vacuum flasks, water bath, refrigerator, beakers, pipettes A special diluent with 12 ingredients including L-glutamic acid, gelatin, antibiotics, and several sugars	Artificial insemination	Clark (1969)
244. Spermatozoa	Fresh bull ejaculate	Bull semen was collected by electroejaculation and pooled. The pooled semen was diluted in egg yolk-buffered citrate glycerol diluent and extended to a final conc of $100x10^6$ live normal spermatozoa/ml. Batches were stored in full, stoppered tubes, insulated with cotton wool, and placed in a vacuum flask with ice to cool to 5°C and equilibrate. 18 hr later the samples were further diluted to $50x10^6$ and then sealed into 1 ml glass ampules and frozen to -70°C using a Linde BF3-2 liq nitrogen biol freezer.	Samples showed no significant loss of quality or fertility after 18 hr storage and transport in stoppered tubes at 5°C.		5 min	18 hr at 5°C	7-8	Test tubes with stoppers, cotton wool, vacuum flask with ice (or refrigerator), ampules, Linde BF3-2 biol freezer, heat sealer Egg yolk-buffered citrate glycerol diluent	Artificial insemination	Singleton (1970)
245. Stomach, intestines and pancreas	Freshly excised canine organs	Dog stomach, intestines, or pancreas were excised by described surgical techniques. The intestines and stomach were cooled to 5°C, while the pancreas was perfused with cold (4°C) balanced salt soln contg 5% low mol wt dextran at pH 7.4. Then the pancreas was cooled to 2°C, kept under 4 atm of oxygen and perfused with autogenous plasma diluted with balanced salt soln at a rate of 20-50 ml/hr.	Stomach and intestines could be maintained for 5 hr and successfully reimplanted. The pancreas with its attached proximal portion of the duodenum could be preserved *in vitro* for 22 hr and reimplanted successfully.	A description of an intestinal transplant from cadaver to human was described in detail. The patient died from complications but the procedure appears to be feasible.	Varied with organ	5 hr for stomach and intestines; 22 hr for pancreas	3-5	Surgical tools, refrigerator, containers, cannulas Autogenous plasma diluted with balanced saline soln	Transplants	Lillehei (1967)

REFRIGERATION (Continued)

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246. Teeth	Freshly excised human teeth, usually premolars	Donor teeth that have incomplete root development with wide open root ends, root length at least equal to the height of the crown, and undamaged periodontal membrane or root sheaths were immediately placed in Hank's soln contg antibiotics. The teeth were then refrigerated at 2°-10°C.	Teeth extd and placed in Hank's soln at 2°-10°C can be stored up to several mon before transplantation. Transplanted teeth required 8-12 wk to become immobile, but no tissue rejection was noted. Transplanted teeth have survived more than 5 yr.		Few min	Several mon before transplanting; transplants have survived over 5 yr	3	Surgical tools, refrigerator Hank's soln contg antibiotics	Transplants	Viener (1969)
247. Thyroid glands	Freshly excised guinea pig thyroid lobes	Guinea pig thyroid lobes were frozen for 15 min at -3° to -5°C or supercooled at -8°C with DMSO protectant. Then they were thawed and incubated at 37°C with I^{131} iodide to test the effect of freezing on metabolism of thyroid tissue.	Freezing leads to the irreversible inhibition of diiodotyrosine formation in thyroid tissue; but thyroid tissue supercooled to -8°C retains its ability to form diiodotyrosine and iodinate thyroglobulin.	Ice crystal formation rather than low temp *per se* was responsible for effects observed in prefrozen glands.	15-20 min	Not given	3-4	Dubnoff incubator, conical flasks, ice-salt bath, water bath Krebs Ringer bicarbonate buffer, pH 7.4 with or without 10% DMSO; $iodide^{131}$; glucose; potassium iodide	Study of thyroid metabolism	Cavalieri (1963)
248. Tympanic membrane	Cadaver drumhead attached to the malleus	Homograft tympanic membrane was obtained from a donor within 12-24 hr after death. The bone contg the drumhead was placed in a preservative soln of 1:5000 aq Cialit (Sodium salt of an organomercurial compd contg sulfur and methylene). The graft was refrigerated until ready for use, or it could also be kept in a tissue bank for up to 3 mon.	Preserved tympanic membranes were still useful for transplants after 3 mon of storage.	The preservative used was bactericidal and nonirritating to living tissue.	24 hr	Up to 3 mon	3-4	Surgical tools, refrigerator, glass container 1:5000 Cialit (sodium salt of an organomercurial compd contg sulfur and methylene)	Transplant for repair of perforated eardrum	Brandow, Jr (1969)
249. Urine	Fresh human urine samples	Urine was obtained from normal subjects and was refrigerated at 5°C for 24 hr. The urine was then pooled, sterilized by pressure filtration, and stored at 5°C in 100 ml aliquots in sterile bottles at pH 7.0. The sterile urine samples were inoculated with a known initial conc of bacteria, and the samples were then stored at 0.5°, 10°, and 15°C. A portion of each sample was plated every 24 hr for 4 days to test for bacterial growth.	Bacteria multiplied rapidly at 15°C, but not at 10°C or lower. Therefore refrigerators holding urine samples should be carefully monitored to keep the temp below 10°C at all times.			4 days	8-9	Refrigerator, pressure filter equipment, sterilizer, pipettes, bottles, Petri dishes Known conc of bacteria, sterile urine 'standard', Trypticase Soy Broth	Detn of bacteriuria	Ryan (1963)

REFRIGERATION (Concluded)

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250. Urine	Fresh urine from patients with urinary tract infections	Clean-voided or catheterized samples of urine which contained red and white blood cells, were obtained from male patients with urinary tract infections and refrigerated immediately; pH, red and white blood cell counts, and microscopic examinations were made within one hr of collection. Cell counts were made at 2 hr intervals the first day, once a day for the next four days, then at longer intervals of time. The same tests were run on aliquots made acid or alk to see what effect hydrogen-ion conc had on blood cell preservation.	Red and white blood cells persisted in refrigerated acid urine of relatively 'normal' specific gravity for at least 10 days; up to 45 days in some instances. Undiluted refrigerated urine that is slightly alk may be examined for blood cells for several hr after collection.	After 24 hr at room temp bacteria which split urea may be present and cause alkalinity to rise and storage conditions to change.	Few min	From 10-45 days	3	Microscope, collection containers, Spencer hemocytometer, refrigerator 0.1 N NaOH, 0.1 HCl	Red and white blood cell counts in urine	McIntyre (1965)
251. Urine	Fresh human urine	Urine samples were stored at a variety of temp and at different pH to find the best storage conditions for preserving gonadotrophic hormone intact.	The optimum pH was 5, and samples should be stored at 20°C. Refrigeration did not prevent the destruction of the hormone.		Few min	6 days	1-2	Containers Buffers	Anal of gonadotrophic hormone	Mazet (1968)
252. Urine casts	Suspension of casts in fresh human urine	Urine at pH 4.0-5.5 contg casts, was placed in conical tubes to which 3-4 drops of 40% formaldehyde had been added to kill yeast and bacteria. The tubes were refrigerated 2 hr, and the resulting supernatant was decanted leaving a sediment contg the casts. Serum of the same blood type as that found in the urine may be used for mounting slides of the casts or for preserving the sediment during storage in the refrigerator.	Urine casts remained stable 4 to 12 mon in the refrigerator. Leukocytes, granular cells, red cells, and nearly all crystals maintained their morphology during this time.	The technique does not work well with highly alk specimens.	2 hr	4-12 mon	3-4	Refrigerator, conical tubes, pipettes, Petri dishes, slides 40% formaldehyde, Sedi-stain (Clay-Adams, Inc, New York), blood serum	Instructional material for students	Lehman (1968)

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LYOPHILIZATION

SPECIMEN DESCRIPTION	ORIGINAL FORM OF SAMPLE	BRIEF DESCRIPTION OF METHOD	LIMITATIONS OF METHOD	OTHER COMMENTS ABOUT METHOD	ESTIMATED PRESERVATION TIME	SURVIVAL TIME	NO. OF STEPS	EQUIPMENT AND REAGENTS USED	END USE OF SPECIMEN	REFERENCE
253. Antigens	Various ext from growing cultures	Brucella abortus, dourine, and glanders antigens were prepared by various methods (outlined). The antigens were titrated, added to 1 ml ampules, and frozen in a rotary device in an alc-dry ice mixt. Then they were lyophilized at -76° to -78°C for 6 hr. Ampules were sealed under vacuum and stored at 4°C until used.	Dourine and glanders antigens maintained their activity for 3 yr. Brucella antigen activity decreased gradually after 9 mon.		6-7 hr	Brucella abortus antigen, 9 mon; Glanders and dourine antigens, 3 yr	5-6	Ampules, rotary flask and motor, alc-dry ice bath, Usifroid freeze-dryer, heat sealer, refrigerator Dry ice, alc	Antigens for use in complement-fixation tests	Grycz (1969)
254. Apples	Fresh apple slices	A freeze-drying technique suitable for lab or pilot plant quantities of foods or other materials was described. This technique used a simple vacuum app along with molecular sieves for the adsorption of water vapor. Samples were first frozen to -20°C on screen trays, then placed between screen trays of molecular sieve adsorbents for drying.	Type 4A molecular sieves used with this app adsorbed 15-18% of water vapor by wt from apples, and were significantly more efficient than activated alumina.	Initial freeze-drying rates of apple slices at 25°C was estimated as 0.1 lb H_2O/sq ft/hr	0.1 lb H_2O/sq ft/hr at 25°C	Not given	5-6	Drying chamber (26 liters); vacuum pump; type 4A molecular sieves, 1/16" pellets (Linde), airtight container for pellets; screen trays; strain gage; thermocouple; electronic recorder; freezing chamber	Freeze-drying foods or lab materials	Saravacos (1967)
255. Arteries	Fresh or cadaver arterial segments	Heterologous arterial grafts taken from cadavers without aseptic precautions were sterilized with ethylene oxide, rapidly frozen in liq nitrogen, dehydrated, and stored at room temp in glass containers sealed under high vacuum.	Successful grafts have been made with vessels stored 2-3 mon, and the author estimated that the vessels would still be useful after 1 yr.	Using ethylene oxide as a sterilant seemed to diminish the antigenicity of the tissue.	8 hr	Up to 1 yr	6-7	High vacuum pump, liq nitrogen specially-designed traps, lyophilizer, glass tubes, surgical tools, oxygen torch Ethylene oxide, physiol saline soln, penicillin, liq nitrogen, dry ice	Tissue grafts	Hufnagel (1954)
256. Ascites	Fresh human ascites fluid	Sterile 300 ml bottles were filled with 150 ml of ascites fluid from a polyethylene tube inserted into the peritoneal cavity. The fluid was frozen at -35°C by rotating the bottle. The vacuum drying process began 14 hr later and took 24 hr. The dried product was dissolved in apyrogenic water before use.	Not given		1½-2 days	Not given	3-4	Lyophilizer, collection bottles, polyethylene tubing, surgical tools Apyrogenic water	Correction of hypoproteinemia in patients with portal hypertension	Klen (1968)

LYOPHILIZATION (Continued)

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257. Bacteria	Bacterial suspensions	Washed cell suspensions resuspended in a small vol of fresh media (10×10^{11} viable cells/ml) were frozen into small pellets at -78°C until used. One vol of thawed pellets was mixed with 49 vol of soln contg 1% oxidizable sugars, and the suspension was freeze-dried in a batch-type dryer at -50°C and 40 μ Hg under unfavorable humidity conditions.	From 5-50% *Serratia marcescens* cells survived freeze-drying in the presence of various sugars. Monosaccharides provided the best protection against freeze-drying. Oligosaccharides provided the best protection against aerosolization. Suspensions survived longer than 72 hr at 4°C.		3-4 hr	>72 hr	5-6	Waffle-iron freeze-dryer with temp control, 15 ml vials Tryptose-glucose phosphate medium, several common sugars (1% soln) distd water	Storage of organisms at unfavorable humidity	Zimmerman (1962)
258. Bacteria	Bacterial suspension in liq media	Arginine, homoargine, argininic acid, and related compounds were tried as suspending media for freeze-drying bacteria. *S. cremoris*, *L. arabinosus*, *M. lysodeikticus*, *E. coli*, and *A. aerogenes* were cultured on various media. Media was adjusted to 0.06 M of the tested compd and kept at pH 7. Cells were harvested at the stationary phase and washed with saline. Washed cells were resuspended in the suspending media to a final conc of $1\text{-}2 \times 10^9$ viable cells/ml. One ml samples were freeze-dried in sterile vials at -30°C with final heating to 30°C after vacuum drying.	From 13-88% of organisms protected by arginine were viable after freeze-drying. Other protectants tried were not quite as effective as arginine.	The α-COOH, α-NH_2 and guanidino groups are all essential to the protective action of arginine, but the optical activity of the molecule is not important. The -NH_2 group can be replaced by an -OH group without affecting activity.	8-10 hr	Not given; probably a long time	3-4	Petri dishes or containers, glass vials, freeze-dryer (Stokes 2003 F-2) Various media, HCl, NaOH, arginine, homoargine, argininic acid	Preserved strains of bacteria for research or further culturing	Morichi (1965a)
259. Bacteria	Bacterial suspensions	DL-Threonine, DL-allothreonine, and DL-tartaric acid as well as the D- and L- forms were added to 14 strains of bacteria. The bacteria were then lyophilized, and the protective effect of the various compd was assessed.	DL-Threonine and DL-allothreonine had a protective effect (between 10 and 70% survival) on bacteria while the D- or the L- form alone had almost none. The optical form of tartaric acid serine, proline, and pyrrolidonecarboxylic acid did not affect the protective action.		Not given	Not given	3-4	Culture bottles, lyophilizer D-, L-, and DL-threonine, allothreonine, tartaric acid, serine, proline, and pyrrolidonecarboxylic acid	Culture maintenance	Morichi (1965)

LYOPHILIZATION (Continued)

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260. Blood serum	Fresh serum from infected and noninfected cattle	4 anti-Brucella sera and 4 normal sera for the complement-fixation test, and 2 positive and 4 negative sera for the agglutination test were used. The complement-fixation and agglutination titers of positive sera were 800 and 400 respectively. Positive sera were obtained from cattle infected with Brucella abortus, and negative sera were obtained from healthy cattle. Serum titer was determined, then samples were stored in 10 ml ampules, frozen in an alc-dry ice mixt, and lyophilized for 2-7 hr. Ampules were sealed and stored at 4°C until used.	Complement-fixation and agglutination tests remained unchanged for 3 yr.	No anticomplementary activity was observed in lyophilized positive and negative sera for the complement fixation test.	6-8 hr (including lyophilization)	3 yr	6-7	Usifroid freeze-dryer, 10 ml ampules, alc-dry ice bath, heat sealer Alc, dry ice	Diagnostics and therapeutics	Grycz (1969a)
261. Bones	Cadaver femur or tibia removed no more than 24 hr postmortem	Bone homografts from cadaver tibia or femur were aseptically removed within 24 hr postmortem and freeze-dried. The grafts were stored in a vacuum at room temp for 32 mon or less. Eighty bone grafts were performed using the preserved materials. Evaluation of the effectiveness of the method and the material was made.	85% of all the grafts were successful. Donor age, donor site, and graft storage time did not seem to be statistically significant.	The criteria used for classification as a successful bone graft were partial incorporation of the graft by the host, and advanced fracture healing.	Not given	Up to 32 mon	3-5	Lyophilizer, vacuum storage container Not given (exptl method was outlined elsewhere)	Bone transplants	Gresham (1964)
262. Bones	Freshly excised cadaver bone	91 cases of the use of freeze-dried bone homografts in cystic defects of the jaw were evaluated to see how many grafts were successful and what problems were encountered with unsuccessful grafts.	18% of the grafts were unsuccessful, and 82% were successful. Follow-up periods ranged from 3 mon to 9½ yr with an av of 33 mon.	Infection was the greatest cause of failure. No rejection phenomena were noted.	Not given	Survived up to 9 yr after grafting		Not given	Jawbone grafts	Marble (1968)

LYOPHILIZATION (Continued)

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263. Brain	Freshly excised brain tissue or biopsy specimens	Slices of brain tissue 1-2 mm thick were dropped in isopentane. They were then freeze-dried by a specially designed app that does not employ diffusion pumps or auxiliary heaters and is capable of drying simultaneously several batches of brain slices.	Activity of five different enzymes was equal to or higher than the activity in sections preserved by standard fixation methods. Too rapid freezing of large sections caused fissures, while thawing the tissue before it was fully dried caused damage from rapid evolution of water vapor.	The degree of dryness of the tissue was indicated by the rate of water evolution, and the temp of the drying tube was not allowed to rise until 12 hr after the rate of water evolution fell below 50 µm Hg/min/gm tissue.	1-2 days	Not given	4-5	Lyophilizer (described) drying tube with wax bed, platinum loop, filter paper, vacuum flasks Isopentane, paraffin, dry ice and acetone, dry ice and ethyl phenyl ether mixt	Fixation of brain tissue for microscopy	Naidoo (1956)
264. Brain and cord	Homogenized rabbit brain and cord	A 10% (w/v) emulsion of normal, adult rabbit brain and cord in phosphate-buffered saline was homogenized at 0^oC with 10% (v/v) trifluorotrichloroethane (Arcton 113). The mixt was centrifuged, and the supernatant fluids were freeze-dried. This substance was resuspended in phosphate-buffered saline and Freund's adjuvant, and was then tested in guinea pigs to see whether exptl allergic encephalitis lesions occurred.	Lyophilized rabbit brain homogenates extd with trifluorotrichloroethane produced no allergic encephalitis lesions in guinea pigs for up to 33 days.	Use of trifluorotrichloroethane in prepn of rabies vaccine from rabbit or sheep brain should eliminate the encephalitogenic factor responsible for serious side reactions.	Several hr	Not given	5-6	Lyophilizer, mixer, containers, centrifuge, ice bath, surgical tools Trifluorotrichloroethane (Arcton 113, ICI), phosphate-buffered saline, Freund's adjuvant	Rabies vaccine prepn	Kaplan (1968)
265. Brain dura mater	Sterile or nonsterile cadaver dura mater	Sterile or nonsterile dura mater from cadavers was divided into pieces, placed in balanced salt soln contg homologous serum and antibiotics, and held at 4^oC until further processing. Nonsterile samples were sterilized by one of several techniques, then all samples were wrapped in perforated cellophane and frozen in bottles at -70^oC. After freezing, the samples were freeze-dried for 3 days. Both chem and radiation sterilants were used successfully.	Lyophilized dura mater was stored at room temp under vacuum seal for up to 10 yr. 98% of dura mater grafts in patients were considered successful.		3 days	Up to 10 yr	6-7	Surgical tools, refrigerator, perforated cellophane, pyrex bottles, fume hood, Van de Graaff generator, plastic bags, freezer, freezer-dryer Balanced salt soln contg homologous serum, penicillin, and streptomycin; ethylene oxide; 1% beta-propiolactone soln	Tissue grafts	Abbott (1970)

LYOPHILIZATION (Continued)

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266. Chickens, Whole body and feces	Whole chicken and fresh droppings with some foreign inclusions such as food and feathers	Whole chickens were weighed, thoroughly minced, freeze-dried and stored at room temp. Chicken droppings were also collected and stored in wash bottles at -20°C until needed. Then the droppings were milled and dried using an acid-scrubbing system attached to the bottle with inlet and outlet tubes that were connected to the oven air vent so that ammonia-free air at 80°-85°C dried the droppings. The dried samples were stored in airtight bottles until analyzed.	Less than 2% whole-body nitrogen was lost after lyophilization and storage of minced chicken at room temp for 56 days. No information was given on length of storage of the droppings.	The difference between nitrogen retention as measured by balance and by body anal methods was 13-16%.	Not given for minced tissue; 3 days for droppings	56 days for minced tissue; not given for droppings	3-4 for each method	Lyophilizer, hand mincer, aluminum trays, glass bottles, Perspex cage for collecting feces, drying oven, ball mill, polythene tubing 0.1 N H_2SO_4, methyl red soln	Total nitrogen anal	Davidson (1968)
267. Chorion and amnion tissue	Human chorion and amnion tissue	Chorion and amnion tissue were freeze-dried on synthetic sponge. The tissue was sterilized in saline-streptomycin-penicillin-Ca gluconate soln for 30 min and used to treat burned surfaces by suturing the graft to the surface.	The freeze-dried material had 'ideal storage possibilities' and was effective for grafts in 2nd degree burns. However, the material was not suitable on deep burns or infected areas.	Chorion tissue was generally more successful than amnion for grafts.	Not given	Not given	3	Sponge, surgical tools, lyophilizer, glass containers Saline-streptomycin-penicillin-Ca gluconate soln	Skin grafts	Dahinterová (1968)
268. Electropherograms	Thin-film electropherograms	Alumina, silica gel or kieselguhr thin-film electropherograms were produced using food coloring. They were dried at 100°-110°C and also freeze-dried by first placing the 20x20 glass plates on a bed of powd dry ice for 10-20 sec and then lyophilizing them in a special coldfinger app (described).	Freeze-drying electropherograms eliminated most zone-migration caused by heat drying.	Freeze-drying provided a simple method for detecting zone-migration whenever the phenomenon was suspected.	½-1 hr	Not given	2-3	Powd dry ice bed, special coldfinger lyophilizer (described)	Drying of electropherograms	Criddle (1965)

LYOPHILIZATION (Continued)

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269. Fascia lata	Freshly extd cadaver fascia lata	Fascia lata was removed from cadavers, using sterile conditions, and put in a sterile container contg merthiolate. Tissue was cut in strips parallel to the direction of fibers. Fat was removed by rubbing the strips with sterile gauze, and the strips were then rolled around 10 cm glass tubes. Several tubes were threaded together and immersed into 90% alc cooled by dry ice. Tubes were unthreaded, placed in sterile test tubes, lyophilized under vacuum, and sealed.	The author implied that tissues can be stored for a long time by this method and still be useful for scleral buckling procedures for retinal detachment.	Fascia lata did not cause necrosis of underlying sclera as other substances did.	Several hr	Not given; long period implied	8-9	Surgical tools, sterile containers, gauze, 10 cm glass tubes, alc bath Merthiolate, 90% ethanol, solid carbon dioxide	Transplants for scleral buckling procedures	Shoukri (1966)
270. Feces	24 hr chicken feces collections	Chicken feces were collected quantitatively each morning in plastic boxes with covers. The boxes were stored in a freezer until used. 120 gm of the feces were freeze-dried and ground on a plate mill for detn of fat and energy content before they were stored in the freezer.	Frozen or freeze-dried samples gave better results for anal because there was less chance of oxidation of fats in the fecal samples.		Freezing, few min; Freeze-drying, several hr	Not given	2-4	Plastic boxes with tops, freezer, lyophilizer, plate mill	Anal of fat, energy, and nitrogen content of feces	Petersen (1968)
271. Fungi	Not given	Previously lyophilized strains of Phycomycetes, Ascomycetes and Fungi Imperfect were tested for viability.	Cultures of all 447 lyophilized fungal strains sporulated as well as, or better than, frequently-transferred agar slant cultures. Lyophiles used ranged from 2-23 yr old.		Not given	Up to 23 yr	Not given	Not given	Maintenance of type cultures	Ellis (1968)

LYOPHILIZATION (Continued)

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272. Heart valves	Human and bullock cadaver valves or aortic cusps	Human and bullock cadaver pulmonary valves and aortic cusps were removed up to 12 hr after death. They were sterilized by incubation at 37°C for 2-3 hr in a soln of β-propiolactone in sodium bicarbonate. The valves were then washed with physiol saline soln and stored in Hank's soln at 4°-10°C. They were tested for sterility and finally lyophilized and stored in glass ampules until used. These valves were used for tympanic membrane repair.	93% of the recipients of homostatic valve grafts had intact drums up to 3½ yr later. Four had perforations, and some had other complications.	When bullock tissue was used for tympanic grafts in humans, no significant antigenic reaction was observed, although some reaction may be present.	Not given	Indefinitely	5-6	Surgical tools, refrigerator, lyophilizer, ampules, vials, incubator β-propiolactone in Na_2HCO_3, physiol saline soln, Hank's soln	Tympanic grafts	Cornish (1968)
273. Leaves	Whole leaves	Leaf material was frozen directly in liq air or nitrogen in a lyophilization vessel. Excess liq was poured out, and the vessel was attached to the lyophilization system described. Several samples were lyophilized at once. The leaf material was then ground in a Wiley mill and stored in stoppered containers until used.	Not given	Drying can also be carried out with a simple dessicator over alumina.	4-5 hr	Indefinitely if kept dry	3	Liq nitrogen, lyophilization app, lyophilization containers, stoppered bottles	Leaf anal	Millikan (1964)
274. Muscle	Fresh slices of beef muscle	Beef longissimus dorsi muscle samples, 1 mm thick, were either quick-frozen in isopentane at -150°C or allowed to freeze in a cold room at -10°C. Slices from these samples were freeze-dried in a constant-temp high vacuum app at -10°C. Some were warmed to 80°C, and a comparison of the effects of heat in the presence of 0.2% and 2% moisture on protein extn was made.	There was no loss in extd protein or increase in insol residue due to freeze-drying if the temp was kept at -10°C. Exposure of freeze-dried structural proteins to 80°C reduced protein solubility by 65%.	Heat, rather than freeze-drying, reduced the extractability of muscle proteins.	~ 24 hr	Not given	3	Vacuum freeze-drier that maintains constant temp, isopentane freezer, specimen chamber, cooling bath, oil bath Isopentane	Study of protein denaturation	MacKenzie (1967)

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275. Muscle	Aged, refrigerated lean meat	Slaughtered beef carcasses were cooled to 3°C, muscles were dissected and aged in plastic bags for on week, fat was trimmed, and the meat was frozen in a -30°C freezer for 1-3 days. Transverse slices of the muscles were freeze-dried in a radiant heat freeze-dryer to residual moisture of 1.0-1.5% of total dry wt. Meat was packaged in gas-tight pouches using various combinations of head-space gases and/or oxygen scavenging systems to see which offered the best preservation.	Lyophilized beef samples stored with a hydrogen-palladium oxygen scavenger and nitrogen headspace gas had a shelf life of 1 yr at 30°C.	Carbon dioxide in the headspace affected reconstitution and organoleptic qualities of meat. The more fat meat had, the harder it was to store it for long periods.		1 yr	9	Freeze-dryer, equipment for packaging under nitrogen Liq nitrogen	Food	Bengtsson (1968a)
276. Muscle	Suspension of fragmented rabbit and lobster sarcoplasmic reticulum	Rabbit and lobster fragmented sarcoplasmic reticulum were homogenized in 0.3 M sucrose and 10 mM Tris maleate, centrifuged, and lyophilized. Dithiothreitol had little effect on Ca uptake, ATPase activity, or increased stability during storage of rabbit white-muscle sarcoplasmic reticulum, although it did prevent loss of activity in lobster samples.	Lyophilized, fragmented white-muscle sarcoplasmic reticulum from rabbits lost no activity after 4 mon of storage. Lyophilized lobster fragmented reticulum was as active as fresh sarcoplasmic reticulum after 18 days of storage.		Few hr	Up to 4 mon	3-4	Lyophilizer, Waring blender, centrifuge, centrifuge bottles Sucrose, Tris maleate buffer, distd water, dithiothreitol, culture medium	Muscle tissue studies	Sreter (1970)
277. Mycoplasma	Suspensions of Mycoplasma	Broth cultures of 26 strains of Mycoplasma were mixed with eq amt of sterile skim milk (10 gm powd/100 ml water). 1 ml samples were lyophilized in 3 ml ampules. Cultures were shell-frozen in alc and dry ice and freeze-dried under vacuum for 5-6 hr. Ampules were sealed and stored in freezers at -26°C, or -65°C, or both. The effects of freezing and thawing on the various strains were noted.	All cultures were viable through 3-4 yr of storage at -65°C, with the greatest loss of viability occurring during the first mon of storage. The cultures were viable up to 10 mon at -26°C. The effects of freezing and thawing varied from strain to strain.		6-7 hr	Up to 4 yr	4-5	Flask shaker, culture plates, ampules, flasks, autoclave, alc-dry ice container, freeze-dryer, oxygen torch, freezer Agar medium contg Difco Heart Infusion agar, Heart Infusion Broth, yeast ext, and Proteose Peptone #3; powd milk; alc; dry ice	Maintenance of cell lines	Kelton (1964)

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278. Nerve growth factor	Venom from commercial suppliers	Nerve growth factor from snake venom of Russell's viper was purified by a chromatographic method (described). The highly active material was stored in phosphate-buffered saline at pH 7.4 or 10.8 at 2°C, or was freeze-dried and stored at 2°C.	Highly active nerve growth factor loses potency on storage in soln. An equivalent response to that given at a conc of 10^{-14} g/ml requires 10^{-10} g/ml after storage in saline at pH 7.4 at a conc of 10 mg/ml for 6 days at 2°C, but this less active material can be stored at pH 10.8, even in very dilute soln, for up to 10 wk without further change. Freeze-dried nerve growth factor is more stable but activity falls to 10^{-10} g/ml after 12 wk at 2°C.	Neither embryo ext nor serum was necessary for the growth of nerve fibers if a small amt of nerve growth factor was present in the culture medium.	Varied with method	Up to 12 wk	4-6	Refrigerator, lyophilizer, containers Phosphate-buffered saline	Stimulation of growth of embryonic sensory and sympathetic ganglia	Banks (1968)
279. Pituitary gland	Acetone powd of bovine pituitary posterior lobes	Acetone powd of bovine pituitary lobes (1.8 I.U. of oxytocic activity/mg) was extd by suspending the powd in a suitable medium. After 18 hr at 4°C the insol material was removed by centrifugation. The supernatant was dialysed against distd water and freeze-dried. The freeze-dried samples were compared with fresh tissue for sol protein content.	Electrophoresis of fresh protein showed 8 distinct constitutents, while electrophoresis of lyophilized samples showed only 6. Cathepsin activity was most active at pH 3-4. For best results cathepsin activity should be destroyed.	This method allows neurosecretory granules to be produced and stored in large amt from a readily available supply of bovine pituitary posterior lobes.	Not given	Not given	6-8	Desiccator, refrigerator, lyophilizer, dialyzer, centrifuge Acetone; distd water; incubation medium contg bovine Hb, acetic acid and $(NH_4)_2SO_4$ at pH 3.5; trichloracetic acid; NaOH	Study of sol proteins in pituitary neurosecretory granules	Dean (1967)
280. Pituitary gland	Live winter <u>Necturus m. maculosus</u>	Hypophyses of thyroidectomized and normal winter <u>Necturi</u> were divided into rostral and caudal portions, and these portions were transferred to coverslips resting on dry ice. Groups of 6 or 12 were weighed in the wet stage and stored at -18°C after lyophilization. The freeze-dried samples were homogenized and injected into mice for bioassay of thyrotrophic activity.	Thyroid-stimulating hormone activity was still intact after freeze-drying. No comparison with nonfreeze-dried specimens was given.	In normal <u>Necturus</u>, nearly two-thirds of the thyroid-stimulating activity was in the rostral portion. After thyroidectomy caudal portions yielded 95% of the thyrotrophic activity.	Not clear	Not given	5-6	Dissecting tools, glass coverslips, powd dry ice, lyophilizer	Thyroid-stimulating hormone assay by the method of McKenzie (1958)	Aplington, Jr (1968)

LYOPHILIZATION (Continued)

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281. Protein,	Wheat leaves	Wheat-leaf protein, extd by the method of Morrison (1961), was lyophilized and stored at -10°C.	Wheat-leaf protein was stored for 16 mon without serious impairment to its nutritive value.	Very little detail was given on the preservation method. The paper dealt mainly with methods of estimating nutritive value of proteins, not long term storage.	Not given	16 mon	3	Extraction app, lyophilizer, refrigerator, flasks	Food protein	Buchanan (1969)
282. Rectal gland	Freshly excised shark rectal gland	The rectal gland of elasmobranchs was removed at the site of catch, frozen on dry ice, and shipped to the laboratory within 1-2 days. Wet wt was recorded, and small samples were taken for dry wt detn and histology. The rest of the gland was homogenized in a 10% (w/v) aq soln. Aliquots were lyophilized at -20°C and stored at -25°C until used for Na-K-activated ATP-ase enzyme studies.	Samples still had high enzyme activity after storage.	Histological specimens were fixed with 10% formalin.	Several hr	Not given	6-7	Surgical tools, dry-ice shipping container, homogenizer, pipettes, lyophilizer, freezer Water	Study of Na-K-activated ATPase in elasmobranch rectal glands	Bonting (1966)
283. Ribosomes	Frozen, minced dog pancreas	Canine pancreatic ribosomes were prepared in the cold by a complex series of suspensions, precipitations by Mg^{++}, and centrifugations (described). Purified ribosomal suspensions were either stored for not more than 2-3 days on ice, or lyophilized and stored at -20°C.	2 mg of ribosomes were obtained per gm of wet tissue. Lyophilization of the postmicrosomal ribosomes caused partial dissociation.		2-3 days	2-3 days on ice	> 10	Tissue homogenizer, gauze, centrifuge, dialyzer Sucrose soln, disodium deoxycholate soln, soybean-trypsin inhibitor, magnesium acetate, potassium phosphate buffer	Study of ribosome properties	Hollinshead (1968)

LYOPHILIZATION (Continued)

SPECIMEN DESCRIPTION	ORIGINAL FORM OF SAMPLE	BRIEF DESCRIPTION OF METHOD	LIMITATIONS OF METHOD	OTHER COMMENTS ABOUT METHOD	ESTIMATED PRESERVATION TIME	SURVIVAL TIME	NO. OF STEPS	EQUIPMENT AND REAGENTS USED	END USE OF SPECIMEN	REFERENCE
284. Saliva	Fresh human saliva	Parotid saliva from adult males was collected by paraffin mastication using modified Lashley cups. Individual or pooled collections of the saliva were dialysed against deionized water at 4°C to remove inorg salts. The salts were then lyophilized. Lyophilized samples were used for zone electrophoresis after being dissolved in tris-EDTA-boric acid buffer to a final conc of 15% (w/v).	None given. Lyophilized saliva seemed to give desired results upon electrophoresis and should have a reasonably long shelf life.		1-2 days	Not given	4-6	Modified Lashley cups, dialyzer, lyophilizer Paraffin, deionized water	Saliva electrophoresis	Meyer (1965)
285. Saliva	Fresh sputum from fasting human donors	Saliva was collected from fasting, healthy donors after the mouth had been rinsed with a bactericidal soln. Saliva was collected in sterile beakers immersed in ice. Each sample was dialyzed against distd water at 4°C for 24 hr, then freeze-dried. In some collections salivary flow was stimulated with a tablet of citric acid. Length of collection time was carefully controlled.	Samples were used to isolate sialogastrone. Use of citric acid stimulation lowered the gastric inhibitory activity of saliva by raising the vol, and apparently by causing an intrinsic change in the content of the saliva.		2 days	Not given	4-5	Sterile beakers, ice bath, dialysis tubing, dialyzer, lyophilizer Bactericidal soln, citric acid	Characterization and isolation of sialogastrone	Menguy (1967)
286. Sclera	Sclera from human enucleated eyes used in keratoplasty	Sclera obtained from enucleated eyes used in keratoplasty were cleaned of fat and episcleral tissue. Intraocular contents were removed, and the inside surfaced rubbed with gauze to remove the uveal pigment. The stump of the optic nerve was cut, and sclera was either cut into straight or zig-zag strips. The strips were placed for 20 min in previously boiled glycerin contg streptomycin. Then they were washed with saline, rolled on thin glass tubes, and stored in larger tubes which were immersed in a mixt of abs alc and dry ice for 20 min, then lyophilized. The tubes were sealed by flame.	Strips can be refrigerated in glycerol-streptomycin soln at 4°C until lyophilized. The lyophilized strips may be used in lid or retinal detachment operations for an 'indefinite period of time'. Sterile conditions were followed throughout.		Several hr		7-8	Surgical tools, surgical gauze, small glass tubes, larger tubes, Edward's vacuum lyophilizer, alc-dry ice bath, heat sealer Boiled glycerin with 1 gm of streptomycin, sterile saline soln, abs alc, dry ice	Tissue repair and transplantation	Shoukry (1966a)

LYOPHILIZATION (Continued)

SPECIMEN DESCRIPTION	ORIGINAL FORM OF SAMPLE	BRIEF DESCRIPTION OF METHOD	LIMITATIONS OF METHOD	OTHER COMMENTS ABOUT METHOD	ESTIMATED PRESERVATION TIME	SURVIVAL TIME	NO. OF STEPS	EQUIPMENT AND REAGENTS USED	END USE OF SPECIMEN	REFERENCE
287. Scorpion venom	Fresh-milked scorpion venom	Approx 450 Vejovis spinigerus scorpions were milked. All samples were lyophilized and stored in the dark at 5°C until used. The chem composition of the venom was investigated during storage.	LD_{50} of the venom slowly changed over 1 yr. In one case LD_{50} fell from less than 5.0 mg to 20 mg/kg body wt of the test animal.		Not given	Kept 1 yr but deteriorated over this time	3-4?	Lyophilizer, containers, refrigerator	Toxicity studies; electrophoresis of proteins	Russell (1968)
288. Spermatozoa	Fresh human, fowl and rabbit semen	Human semen was mixed with an eq vol of 10% glycerol in Baker's medium, while fowl semen was mixed with from 10-40% glycerol in Ringer's soln. The fowl semen samples were frozen to -79°C, and some were lyophilized at -25°C.	50% motile spermatozoa were recovered from freeze-dried fowl semen that had been frozen to -79°C in the presence of 30% glycerol in Ringer's soln and dried at -25°C. Glycerol also improved the survival rate of human spermatozoa that were frozen but was toxic to rabbit spermatozoa.	Glycerol in conc form was toxic to spermatozoa at room temp.	Several hr	10 wk	Varied	Flasks, freezer, lyophilizer Glycerol, Ringer's soln, Baker's fluid, propylene glycol, ethylene glycol	Artificial insemination	Polge (1949)
289. Spores	Bacterial spores in 5% sugar soln	0.1 ml samples of bacterial spores in 5% soln of glucose, lactose, or fructose were freeze-dried at 0.01 mm of mercury over phosphorus pentoxide for 16 hr. Then they were irradiated with up to $5x10^4$ rads of gamma radiation. The effects of freeze-drying on amt of radiation damage was observed.	Bacterial spores freeze-dried from sugar soln had 5% viability after irradiation with $4x10^4$ rads of gamma radiation.	Protection was apparently associated with the production of a 'glass' during the freeze-drying process. Maltose did not form a glass so it was not effective.	16 hr	Not given	2	Lyophilization flask, vacuum pump, dessicator radiation source Glucose, fructose, lactose, maltose	Radiation protection studies	Cook (1963)
290. Spores	Bacterial spores suspended in culture medium	The effects of water activity, solutes, and temp on the viability and heat resistance of Bacillus and Clostridium strains were investigated.	No loss of viability occurred in freeze-dried bacterial spores after storage at 25°C with water activity of 0.2-0.8. At 0.00 water activity all spores showed loss of viability both in air or under vacuum. Bacillus spores stored at 1.00 water activity lost viability under vacuum but not in air; the reverse was true for Clostridia. Protective agents had variable effects on viability.		Several hr	Up to 6 yr	5-6	Centrifuge, drying oven, pyrex tubes, cotton plugs, lyophilizer, ampules, heat sealer, vacuum chamber Salt soln, NaOH, phosphate buffer	Maintenance of strain lines	Marshall (1963)

LYOPHILIZATION (Continued)

SPECIMEN DESCRIPTION	ORIGINAL FORM OF SAMPLE	BRIEF DESCRIPTION OF METHOD	LIMITATIONS OF METHOD	OTHER COMMENTS ABOUT METHOD	ESTIMATED PRESERVATION TIME	SURVIVAL TIME	NO. OF STEPS	EQUIPMENT AND REAGENTS USED	END USE OF SPECIMEN	REFERENCE
291. Streptomyces	Bacterial suspension	Sterile filter-paper wicks, 5 mm wide, were inserted into 1 ml of a crude suspension of 10% (w/v) Bacto Skim Milk soln and Streptomyces organisms (scraped with a loop from the surface of Hopwood medium). The wicks were each placed in a sterile ampule, and the neck of each ampule was constricted to ½ its original size and freeze-dried for 4 hr. Ampules were sealed and stored.	No apparent loss of viability was noted after 10 mon.	Poor or nonsporulating strains can be preserved with this method. The method is fast (48 samples can be dried at once), and it is particularly useful for preserving genetically marked stocks of Streptomyces.	4 hr	> 10 mon	7	Autoclave, filter paper wicks, glass ampules, cans for drying, freeze dryer (Edwards High Vacuum Ltd), high frequency tester (Model T2, Edwards High Vacuum Ltd), flamemaster double tipping attachment (Buck & Hickman, Ltd), cultures tubes, Petri dishes Bacto Skim Milk, Hopwood medium	Bacterial culture maintenance	Hopwood (1969)
292. Tapeworm scolices	Fluid from hydatid cysts contg tapeworm scolices	Fluid was withdrawn from fresh hydatid cysts and refrigerated 24 hr. The tapeworm scolices were removed from the supernatant and washed 3x in physiol saline by gentle centrifugation and suspension. 4% sucrose soln was added to the final deposit, and the suspension was freeze-dried and stored in ampules under vacuum at 2^{o}-4^{o}C. The preserved scolices were then used to produce antigen for the fluorescent-antibody test. 3% dextran may also be added to the suspending medium to further increase the stability of the lyophilized scolices.	The whole-scolex complement fixation test using freeze-dried scolices gave identical results with frozen antigen. Freeze-dried scolices also gave good reproducible results on the fluorescent-antibody test for diagnosis of human hydatid disease.		2 days	Not given	7-8	Hypodermic syringes, refrigerator, containers, centrifuge, lyophilizer, ampules, vacuum dessicator 4% sucorse soln, 3% dextran soln, physiol saline soln	Hydatid antigen production	Beggs (1970)
293. Tissue culture cells	Cultured mouse embryo fibroblasts	Non-confluent layers of 3T3 cells were prepared in tissue culture dishes and were either lyophilized or irradiated in situ. Fresh 3T3 cells were then added to these prepared layers and their growth followed with radioactive labels.	No growth of added cells occurred on confluent layers of either untreated or irradiated cells. Growth was unimpeded on monolayers of lyophilized cells. When cells were added to non-confluent normal or irradiated cells, the added cells grew until they contacted the pre-existing cells and covered the remaining surface of the dish.	No definite conclusion was drawn as to whether the phenomena of contact inhibition was a phys and/or a chem one.	Few days		Varied with procedure; several	Plastic Petri dishes, dessicator, Van de Graaff generator, incubator Modified Eagle's medium, fetal calf serum, penicillin, streptomycin, buffered saline, dry ice	Investigation of the phenomena of contact inhibition in cell cultures	Schutz (1968)

LYOPHILIZATION (Continued)

SPECIMEN DESCRIPTION	ORIGINAL FORM OF SAMPLE	BRIEF DESCRIPTION OF METHOD	LIMITATIONS OF METHOD	OTHER COMMENTS ABOUT METHOD	ESTIMATED PRESERVATION TIME	SURVIVAL TIME	NO. OF STEPS	EQUIPMENT AND REAGENTS USED	END USE OF SPECIMEN	REFERENCE
294. Tumor cells	Milky ascites fluid from mouse peritoneum	Ehrlich ascites tumors from mouse peritoneal cavity, contg 170×10^6 cells/ml, were diluted two-fold with Hank's soln. One-half ml of this soln was mixed with 0.5 ml each of isotonic sodium glutamate, glycerol, sucrose, or lactose soln. These mixt were frozen rapidly to -22°C and lyophilized 3-4 hr. Various methods of drying, other than by vacuum, were investigated also and results reported.	10% sodium glutamate proved to be the best medium for lyophilization. Av survival time of mice inoculated with lyophilized cells was doubled over that of controls, thus indicating that only a few cells survive the treatment.	Cells from milky white ascites survived lyophilization better than those from hemorrhagic ascites.	4-6 hr	Not given	5-6	Lyophilizer, freezer, ampules, air dryer, dessicator, Petri dishes, pipettes Hank's soln, physiol saline soln, isotonic sodium glutamate, glycerol, sucrose, lactose, nitrogen	Tumor studies	Ebina (1968)
295. Vaccine, *Brucella abortus*	Continuous-culture harvest conc diluted to 6% cell conc by packed cell vol	*Brucella abortus* cells from continuous culture were flask-lyophilized to remove impurities, mixed with a freeze-drying soln consisting of Bacto-Casitone (Difco), 5; sucrose, 10; and oxoid monosodium glutamate, 2 (w/v) in distd water at pH 7.0 and dried in bottles on trays in a precooled commercial freeze-dryer. Samples bottles were tested for purity, number of viable organisms, and moisture content. Then the bottles were stored at 4°C.	Lyophilized cells had a 55-60% survival rate; however, rough-celled strains sometimes evolved from cultures of the lyophilized organisms.		36 hr	Not given	7-8	Autoclave, liter aspirator, freeze-drying tray, 5 ml bottles, stoppers, test tubes, Model SMIR freeze-dryer (Usifroid, Paris), refrigerator Bacto-Casitone (Difco), 5; sucrose, 10; oxoid monosodium glutamate, 2, in distd water at pH 7.0	*Brucella abortus* vaccine	Boyce (1966)
296. Vaccines, *Shigella*	Freshly cultured bacteria	Two freeze-dried *Shigella flexneri* 2a strains, a mutant and a hybrid, were rehydrated in Brain Heart Infusion and streaked on agar plates. Colonies were emulsified with the medium and transferred to a seed bottle contg 600 ml of the same medium. After incubation the seed culture was spread over Kolle flasks and incubated again. The organisms were harvested and pooled, and 10 ml portions of the viable organisms were freeze-dried in vaccine bottles at -44°C. The bottles were sealed and stored at 4°C.	Both strains retained the biol properties of freshly grown bacteria for all tests taken after 2 yr storage.	The mutant strain produced a better vaccine than the hybrid strain.	42 hr	Up to 2 yr	10-11	Agar plates, incubator, seed bottles, Kolle flasks, rake, vaccine bottles, stoppers, trays, freeze-dryer chamber, aluminum seals, Tesla coil, refrigerator Brain Heart Infusion (Difco), agar	Preservation of *Shigella* vaccines	Formal (1967)

LYOPHILIZATION (Continued)

SPECIMEN DESCRIPTION	ORIGINAL FORM OF SAMPLE	BRIEF DESCRIPTION OF METHOD	LIMITATIONS OF METHOD	OTHER COMMENTS ABOUT METHOD	ESTIMATED PRESERVATION TIME	SURVIVAL TIME	NO. OF STEPS	EQUIPMENT AND REAGENTS USED	END USE OF SPECIMEN	REFERENCE
297. Viruses	Viruses in tissue culture media	Para-influenza viruses, enteroviruses, rhinoviruses, and respiratory syncytial virus in tissue culture were mixed with equal amt of a stabilizer such as skim milk, glucose, or dextran. 0.1 ml aliquots were put in ampules and dried for one day on a centrifugal freeze-dryer and were sealed at a final vacuum of less than 20 torr. Ampules were stored in an ordinary refrigerator at $4^{o}C$.	A drop of 2 log_{10} units in titre was noted for most viruses tested, but residual infectivity was unchanged after 2 wk at room temp or several mon at $4^{o}C$. Strain differences occurred so the method must be adapted to each organism.	This method maintained viruses in an infectious state for long periods of time as long as stabilizers such as glucose, dextran, albumin, etc, were present. The stabilizers were strain specific.	1-1½ days	2 wk to several mon		Centrifugal freeze-dryer (Edwards model 5PS), ampules, refrigerator, vacuum sealer Skimmed milk, glucose, dextran, culture media	Maintenance of infectious viral cultures	Tyrrell (1965)
298. Viruses	Liq suspension of influenza virus	Influenza virus, strain PR8, in physiol saline soln plus 1% calcium lactobionate and 1% serum albumin was frozen to $-30^{o}C$, slowly brought back to $0^{o}C$, and finally lyophilized at that temp to a moisture content of 3.2, 2.1, 1.7, 1, or 0.4%. The stability of the several suspensions was determined by an accelerated storage test.	Stability (time for dried prepn to lose 1 log of infectivity titer) was greatest at 1.7% moisture. The suspension took 20.2 days at $28^{o}C$ to lose 1 log titer of stability with residual moisture of 1.7%.	The results were inconclusive.	Approx 36 hr	Not given	3	Edwards High Vacuum freeze-dryer, glass vials, freezer Physiol saline with 1% calcium lactobionate and 1% serum albumin	Study of residual moisture effects on lyophilization of influenza virus	Greiff (1968)
299. Yeast	Yeast cells were disintegrated and the extract mixed with NaCl to a final soln of 0.9%. To this suspension was added the various preservatives to be tested.	DMSO, glycerol, sucrose, PVP, and glucose were added to <u>Saccharomyces cerevisiae</u> cells suspended in 0.9% sodium chloride soln, and the suspensions were frozen. The same compd were again added to <u>S. cerevisiae</u> and the samples were dried in an attempt to discover whether freezing or drying causes damage to the organism. Results indicated that drying from the liq state is usually lethal to cells. In drying from the frozen stage, the lower the drying temp the better the survival. DMSO was the best protective agent for <u>S. cerevisiae</u> followed by the others in the order listed above.	No long term effects were studied. Emphasis was on separation of effects of freezing and drying in order to better understand the phenomena.		Not given	Not given	4	Two-stage 'Frigiston' refrigerator mounted on a brass plate under vacuum, Megavac pump, moisture trap, ampules, freeze press DMSO, glycerol, sucrose, PVP, glucose, sodium chloride soln, bovine albumin soln	Study of the effects of freezing and drying on yeast cells	Greaves (1965)

LYOPHILIZATION (Concluded)

SPECIMEN DESCRIPTION	ORIGINAL FORM OF SAMPLE	BRIEF DESCRIPTION OF METHOD	LIMITATIONS OF METHOD	OTHER COMMENTS ABOUT METHOD	ESTIMATED PRESERVATION TIME	SURVIVAL TIME	NO. OF STEPS	EQUIPMENT AND REAGENTS USED	END USE OF SPECIMEN	REFERENCE
300. Yeast	Freshly cultured yeast cells	Candida pseudotropicalis was maintained at 15°C on slants of Difco yeast ext, $(NH_4)_2SO_4$, and KH_2PO_4 plus glucose and agar. Slant organisms were transferred to a roller tube contg 10 ml of medium (with galactose instead of glucose) and allowed to grow for 8-20 hr at 37°C. Tube contents were then transferred to a 4 l flask and shaken. Next they were transferred to a carboy, aerated, and grown for 24 hr at 37°C. The yeast cells were collected in a supercentrifuge and washed 10x. The cell paste was frozen, freeze-dried, and stored at -10°C.	Yeast cell paste could be stored for 6 mon and still be suitable for extn of uridine diphosphate galactose 4-epimerase enzyme.		Several days	At least 6 mon	>10	Slant culture tubes, autoclave, roller tube, 4 l flask, shaker, aerator, spectrophotometer, Sharples centrifuge Glucose, agar, Difco yeast ext, $(NH_4)_2SO_4$, KH_2PO_4, galactose, Dow Antifoam	Yeast enzyme studies	Darrow (1968)

CHEMICAL PRESERVATION

SPECIMEN DESCRIPTION	ORIGINAL FORM OF SAMPLE	BRIEF DESCRIPTION OF METHOD	LIMITATIONS OF METHOD	OTHER COMMENTS ABOUT METHOD	ESTIMATED PRESERVATION TIME	SURVIVAL TIME	NO. OF STEPS	EQUIPMENT AND REAGENTS USED	END USE OF SPECIMEN	REFERENCE
301. Adrenal glands	Bovine adrenal gland from a freshly killed animal	Bovine adrenal glands were removed 15 min after the death of the animal and kept in ice for 30-60 min until perfusion was begun. The cortex was removed, and the medulla was cannulated through the adrenal vein and tied with a ligature around a small amt of aortic and cortical tissue left. The organ was perfused with a special perfusate (described). Methylene blue was included just before the perfusion fluid entered the tissue. This showed that the cortical tissue was not perfused. Secretagogues were added to the perfusion fluid in an attempt to see whether the medulla reacts differently alone than it does with the cortex attached.	There appears to be no difference in the mechanism for catecholamine secretion from cortex-free perfused medulla as compared to the whole gland.		< 1 hr	Not given	7-8	Surgical tools, ice bath, cannulas, hypodermic syringe, oxygenator, Harvard infusion pump Perfusate contg NaCl, KCl, $CaCl_2$, NaH_2PO_4, $MgCl_2$, $NaHCO_3$ and glucose; methylene blue; misc secretagogues	To study the differences in secretion between the adrenal medulla and the whole gland	Schneider (1969)
302. Animal and plant specimens	Fresh plant or animal tissue	A method for preserving plant and animal tissues so that they retained their natural color and shape and did not deteriorate was presented. The method consisted of treating the tissue with a water-dissolving, volatile, org liq (such as tertiary butyl alc) contg an oxidation inhibitor thiourea and a preserving substance such as sodium sulfite which controls the pH and stabilizes the color and composition of the tissue. After this treatment the specimens were dipped or imbedded in plastic, or treated with wax coatings.	Specimens treated in this manner did not deteriorate for long periods of time at room temp. It was important to use an antioxidant which would protect against oxidation but would not bleach the specimen. Also, adjustment of the pH to approx that of the original specimen was necessary.		Several hr	Not given	Varied	Many reagents were used depending on the type of tissue being preserved	Biol specimens or museum specimens	Fessenden (1953)
303. Animal specimens	Human cadaver	A water-soluble gel embalming fluid contg from 5-60% formalin and cellulose ester gels was used to embalm cadavers.	The gel allowed the embalming fluid to stay in contact with the cadaver, but it could easily be removed with water. Evaporation and excessive fumes were also avoided by the use of a gel.		15 min-1 hr	Not given	2	Water-sol gel embalming fluid contg 5-60% formalin and cellulose ester gels	Embalming	Hayden (1966)

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304. Animal specimens	Freshly killed or dissected animal specimens	The δ-lactone of 2-p-dioxanone was dissolved in an appropriate solvent such as water, benzene, toluene, xylene, dioxane, etc, and used as an embalming agent for animal specimens. The soln was either injected or infused into the specimen, usually through the circulatory system.	Storage from 1 mon to an indefinite period can be achieved by treatment of the specimen with 5-50% of its wt with 2-p-dioxanone compds. Dog hearts remained soft and natural-appearing for 10 mon without signs of deterioration, and rabbit liver was kept 2 mon without deterioration.		Few min after prepn of sample	From 1 mon to several mon depending on specimen	2-4	Equipment varied 2-p-dioxanone in a variety of solvents	Museum specimens	Langner (1966)
305. Arteries	Freshly extd bovine carotid arteries	Freshly extd bovine carotid arteries were immersed in cold water and shipped to labs where they were washed, cleaned, and the tributaries were ligated with 000 surgical silk. Then they were soaked 2½ hr at 37°C in a 1% soln of ficin at pH 5.5. Enzyme action was stopped by soaking in a 1% aq soln of sodium chlorite for 18 hr. The arteries were tanned for 24 hr in 1.3% aq dialdehyde starch soln at pH 8.8. The vessels were washed, tested for strength and leakage, slid onto glass mandrels, and sterilized in 50% ethanol-1% propylene oxide soln for 14 days. The glass container served as a package until used.	Excellent 'patency' occurred with grafts up to 14 days old, when the grafts were applied to blood vessels of similar size; it was not clear how long samples could be maintained in their sterilized form.	There was no antigenic response or rejection of the grafts. They were generally tolerated much better than synthetic materials. The major hazard involved with their use was aneurysm.	About 18 days (including sterilization)	Not clear, longer than 14 days	8	Glass mandrels, sterilizer, glass containers, surgical silk, air under pressure 1% soln of ficin buffered to 5.5 with citrate, 1.3% aq dialdehyde starch soln buffered to pH 8.8 with satd sodium bicarbonate, 1% soln 50% ethanol plus 1% propylene oxide, 1% soln sodium chlorite	Blood vessel transplants	Dale (1969)
306. Bacteria	Bacterial cultures of Thiobacilli and Ferrobacilli	Thiobacilli and Ferrobacilli, two types of bacteria that cause disintegration and loss of large numbers of pyritic mineralogical and fossil specimens in museums, were cultured and treated with a number of volatile sterilants. 4-chloro-m-cresol crystals proved to be the most effective, 4-chloro-3,5-xylenol or a combination of thymol and dicyclohexylamine nitrate (unmixed) both acceptable alternates.	Although extensive field tests over a long period of time have not been performed, the authors feel that crystals of the sterilants mentioned placed in museum cases or containers should inhibit specimen attack by iron and sulfur bacteria. The compds were volatile but relatively nontoxic.		Few min	Not given	Varied	Incubator, cotton wool-plugged flasks, aluminum cups Sterile media	Preservation of pyritic museum specimens	Booth (1970)

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307. Bacteriophage	Suspension of Staphylococcus infected with bacteriophage	A study was made of the effects of phys and chem agents such as thermal inactivation, ultrasonic vibration, lyophilization, long term storage, temp, UV light, salt soln, and soln at various pH on Staphylococcus bacteriophage. Tryptose phosphate broth was used to grow the host organism and was also used as the diluent. Agar was added for plate or slant production.	Bacteriophage Pl was inactivated by heat at a logarithmic rate and has a temp characteristic of 100,000 cal/mole. Inactivation by high frequency oscillation was a first order reaction with a velocity constant of 0.77 cm^3/min. Photoreactivation occurred when U V-treated Pl were exposed to strong visible light. Adsorption constants of Pl to host bacterium SK9 range from 489×10^{-12} at 1^oC to 748×10^{-11} cm^3/min at 37^oC. Pl is most stable at pH 5-7. Mono- and divalent ions tested did not stabilize the phage, and citrate did not prevent phage multiplication. Pl phage in tryptose phosphate broth are stable for 1 mon at 4^oC. Pl can be lyophilized but with a considerable loss of titer.		Varied with method	1 mon at 4^oC	Varied with method	Culture tubes, Petri dishes, autoclave, centrifuge, refrigerator, lyophilizer, Selas filtration candle, alc-dry ice bath, various containers, sonic oscillator (Raytheon), constant-temp water bath, GE germicidal lamp Tryptose phosphate broth, agar, alc, dry ice, physiol saline soln, NaCl, glucose, Na_2HPO_4, HCl, NaOH, sodium citrate, divalent salts, deionized water	To study the effects of phys and chem agents on bacteriophage viability	Holmes (1956)
308. Blood	Fresh human blood	One drop of a satd soln of potassium fluoride was added to each 5 cc of fresh blood. Specimens were collected in tubes contg potassium fluoride soln which had been stoppered with cotton and autoclaved. Samples were kept at room temp.	Blood sugar or carbon dioxide levels in preserved blood samples did not change for up to 7 days at room temp.	Oxalate was not needed if potassium fluoride was used.	Few min	Up to 7 days	3	Test tubes, autoclave, pipettes, cotton plugs Potassium fluoride satd soln	Blood sugar anal by the Folin method, and CO_2 anal by the Van Slyke method	Major (1923)

CHEMICAL PRESERVATION (Continued)

SPECIMEN DESCRIPTION	ORIGINAL FORM OF SAMPLE	BRIEF DESCRIPTION OF METHOD	LIMITATIONS OF METHOD	OTHER COMMENTS ABOUT METHOD	ESTIMATED PRESERVATION TIME	SURVIVAL TIME	NO. OF STEPS	EQUIPMENT AND REAGENTS USED	END USE OF SPECIMEN	REFERENCE
309. Blood	Fresh human blood drawn by venipuncture	The effect of blood storage methods on the prothrombin time measurement of blood by the Quick method was investigated. Oxalated blood specimens were stored in opened test tubes, in stoppered tubes, and in filled Vacutainer tubes. The effects of loss of CO_2, and resultant change in pH, on storage stability were discussed.	Stability of prothrombin time was demonstrated if the oxalated blood specimen was stored in a stoppered, filled tube in a test tube rack at room temp. Vacutainer tubes gave the best results, but it was necessary to fill them full. After 24 hr of storage only 3 out of 86 revealed changes of 2 sec or greater.		$\frac{1}{2}$ hr	24 hr	3-5	Vacutainer tubes, test tubes, needles, centrifuge, test tube rack 0.1 M sodium oxalate soln	Anal of prothrombin time by the Quick method	Schoen (1962)
310. Blood	Freshly drawn citrated human blood	Citrated blood diluted with 5% glucose in a transfusion set leads to clumping or agglomeration of the blood. To try to alleviate the agglomeration the following were tried: 1) NaOH was added to the glucose soln to neutralize HCl, 2) physiol saline and 5, 10, or 25% blood were used instead of glucose 3) 0.9% physiol saline was used to resuspend the clumps 4) donor or recipient plasma was used to dissolve the clumps. All suspensions were examined microscopically for clumping.	Clumping was most pronounced at low conc of blood and at room temp rather than at 37°C. NaOH did not reduce clumping. When blood was diluted with physiol saline, no clumping was observed. Some clumps were formed in 25% blood mixt, but more formed in 5% blood mixt. Clumps can be broken up by vigorous shaking.	No conclusive results were found. The clumping phenomenon did not appear to have serious adverse effects but could cause an embolism if not dissolved.				HETO blood warmer, autoclave Isotonic glucose soln, NaOH soln, blood, blood plasma, physiol saline soln	Transfusion without clumping	LaCour (1970)
311. Blood	Human autopsy blood	Investigation of glucose and ethyl alc content of autopsy blood showed that, if blood glucose was high, microorganisms in the autopsy blood would produce alc even in the presence of 0.5% sodium fluoride.	1% sodium fluoride was necessary to act as both an enzyme and microbial inhibitor in autopsy blood. Blood samples could be kept up to 7 days at 20°-25°C if stored in 1% sodium fluoride.	The Folin Wu method of glucose anal was not satisfactory for autopsy blood glucose anal.	Few min	7 days at 20°-25°C	2	Blood collection app, sterile containers 1% sodium fluoride soln	Glucose and ethyl alc anal	Plueckhahn (1970)

CHEMICAL PRESERVATION (Continued)

SPECIMEN DESCRIPTION	ORIGINAL FORM OF SAMPLE	BRIEF DESCRIPTION OF METHOD	LIMITATIONS OF METHOD	OTHER COMMENTS ABOUT METHOD	ESTIMATED PRESERVATION TIME	SURVIVAL TIME	NO. OF STEPS	EQUIPMENT AND REAGENTS USED	END USE OF SPECIMEN	REFERENCE
312. Blood cells, Red,	Freshly drawn pig blood	Preservability of pig red blood cells was examined in citrate and Alsever's soln completed with various quantities of KCN or NaCN. Blood was collected from unsterile skin by a hypodermic syringe contg a small amt of sodium citrate and was put at once into 4 ml of citrate soln. Red blood cells were washed twice before being suspended in the test soln.	Red blood cells could be preserved in citrate soln contg 1 part cyanide to 17,000-21,000 parts red cell-citrate mixt for 4 wk. 2,000 and 3,000 parts of red blood cells in Alsever's soln to 1 part cyanide lasted 60 days in excellent condition.		Few min	Up to 60 days	4-5	Hypodermic syringe, centrifuge, test tubes Sodium citrate soln, Alsever's soln, KCN or NaCN (various dilutions)	Blood-typing standards	Fésüs (1969)
313. Blood cells, Red	Aq soln of rabbit red blood cells contg 0.6% sodium chloride to prevent simple osmotic hemolysis	Ten chemical antimicrobial preservatives were compared for hemolysis of red blood cells. Addition of 10-20% DMSO dramatically decreased the hemolytic activity of all the antimicrobials. Preservatives covered were: Phenol, benzyl alc, phenyl ethyl alc, m-cresol, chlorobutanol, p-chlorophenol, thimerosal, chlorhexidine diacetate, benzalkanium chloride, and phenylmercuric nitrate.	Denaturation of blood occurred at about 30% DMSO.		Few min	Not given	4	Centrifuge, test tubes, water bath, pipettes DMSO, several antimicrobials (listed), 0.6% sodium chloride, physiol saline soln	Hemolysis prevention	Ansel (1970)
314. Blood cells, Red	Freshly collected human blood	Red cells lost their biconcavity and progressive shape changes occurred on loss of ATP during storage. This paper investigated the effect of adenine on these phenomena in blood collected in ACD at 37°C.	Blood cell vol and mean cellular Hb remained constant 45 hr at 37°C, then large shrinkage of the cells occurred coinciding with loss of ATP. Also at this time K^+ was exchanged for Na^+ within the cells. These phenomena were completed in 60 hr without adenine, but were prolonged to 90 hr with the addition of adenine	Contraction of cells, ATP depletion, and cation equilibration were accelerated in blood collected in heparin, fluoride, and in cells suspended in 0.15 M NaCl.	Few min	90 hr	3	Plastic bags, blood collection app, transfer packs, water bath, plastic tubing ACD (NIH formula A), adenine	Adenine effect on red blood cell vol and metabolism	Lionetti (1970)

CHEMICAL PRESERVATION (Continued)

SPECIMEN DESCRIPTION	ORIGINAL FORM OF SAMPLE	BRIEF DESCRIPTION OF METHOD	LIMITATIONS OF METHOD	OTHER COMMENTS ABOUT METHOD	ESTIMATED PRESERVATION TIME	SURVIVAL TIME	NO. OF STEPS	EQUIPMENT AND REAGENTS USED	END USE OF SPECIMEN	REFERENCE
315. Blood plasma,	Freshly drawn human blood	The effect of heparin on potassium conc of blood plasma was studied. Various levels of heparin were added to blood samples and potassium was analyzed.	200-300 units of heparin/2-5 ml of blood had a stabilizing effect on plasma potassium values. Heparinized plasma can be left in contact with blood cells for more than 4 hr with no change in potassium conc.	The principal error in plasma potassium anal was due to the muscular work of the arm from which the venous blood was taken for anal.	Few min	4 hr	Not clear	Test tubes, centrifuge, pipettes Heparin	Plasma potassium anal	Hultman (1962)
316. Blood serum	Dog and human blood freshly drawn by venipuncture; rat blood collected by severance of the subclavian artery	Blood samples from humans, dogs, and rats were added to tubes with no anticoagulant, or with 25 mg of heparin, 15 mg of potassium oxalate, or 25 mg of sodium citrate. Serum and plasma were separated and stored at 2°C until used. The effects of the various anticoagulants on serum and plasma amylase activity were measured.	Amylase activity in human blood sera was almost 20% lower in the presence of oxalate or citrate than it was in controls, whereas heparin had no significant effect on amylase activity. Amylase activity of rat and dog blood sera were not appreciably changed by any of the anticoagulants. Heparin did not change human saliva amylase activity either.	Dog and rat amylases appear to be different from human amylase in that their activity was not influenced by calcium.	Few min	Not given	2-3	Syringes, surgical tools, test tubes, refrigerator Heparin, sodium citrate, potassium oxalate, heparin substitute (mannuronic acid deriv)	Anal of amylase activity by the method of Van Loon (1952)	McGeachin (1957)
317. Blood serum	Unadulterated human blood serum	Citrate buffer, 0.5 ml of 0.2 M soln, pH 5.2, was added to test tubes and dried at 75°C. When serum, 1.50-1.75 ml, was added to the tubes, the citrate buffer redissolved. This method eliminated error caused by adding incorrect vol of acid soln.	Serum acid phosphatase activity was unchanged up to 48 hr using this method.	Ordinarily a liq acid solution is used and allows more room for error.	Few min	> 48 hr	3-4	Pipettes, test tubes 0.2 M citrate buffer	Clinical anal of serum acid phosphatase	Romslo (1970)

CHEMICAL PRESERVATION (Continued)

SPECIMEN DESCRIPTION	ORIGINAL FORM OF SAMPLE	BRIEF DESCRIPTION OF METHOD	LIMITATIONS OF METHOD	OTHER COMMENTS ABOUT METHOD	ESTIMATED PRESERVATION TIME	SURVIVAL TIME	NO. OF STEPS	EQUIPMENT AND REAGENTS USED	END USE OF SPECIMEN	REFERENCE
318. Blood substitute	Excised heart (animal species not given)	A mixt of dried bullock blood dissolved in water to resemble normal blood was diluted with 5 parts 0.75% physiol saline soln. This was used as a normal blood 'standard' to perfuse the ventricle of an excised heart. Soln of distd water or 0.75% physiol saline soln to which egg white, serum albumin, potassium chloride, other potassium salts, syrup (glucose?), sodium phosphate and/or more sodium chloride were added, were perfused through the ventricle, and the results were compared to the 'standard' for effects on ventricular systole and diastole.	0.75% physiol saline soln to which 1×10^{-4} parts of potassium chloride have been added made an excellent circulating fluid for detached heart expt according to the author.	This was Ringer's original paper.	Varied	Up to 4½ hr	Varied	Cannulas, Roy's tonometer 0.75% physiol saline soln, egg white, serum albumin, dried bullock blood, distd water, potassium chloride, several potassium salts, syrup, sodium phosphate, sodium chloride	Blood substitute for heart perfusion	Ringer (1882)
319. Bone	Freshly excised human cadaver bone	Anorganic bone prepared by treating ordinary bone with hot ethylenediamine to remove the org matter, was implanted into the tibia of a sheep and into the skull of a rabbit. Its properties were compared to controls and to implants with other commercial bone prepn that did not have the org matter removed.	Anorganic bone may be stored for a very long time, is well tolerated, is a useful space filler, and shows good osteoconduction. It does not have mechanical strength and is replaced in the body very slowly. Kiel bone and Boplant showed evidence of antigenic reaction which anorganic bone did not.	The author mentioned other work where the recipient's own bone marrow was combined with the anorganic bone to give better results.	Not given	Unlimited if kept sealed	3	Surgical tools, sealed containers Ethylenediamine, Kiel bone, Boplant (Squibb)	Transplants	Kramer (1968)
320. Bone	Whole animal skeletons	Animals were skinned, most of the flesh was removed, and the skeleton was boiled in water until the remaining meat was cooked. The meat was removed, and the bones were brought to a boil again. Sodium perborate was added to the boiling water in an amt sufficient to make a 2.5% (w/v) soln. After several hr in this soln the bones were removed, washed in hot detergent soln, and dried.	Frothing took place when perborate was added to the water, therefore the container had to be much larger than the skeleton. This technique of preserving skeletons not only bleached the bones, but it also degreased them.	The conc of perborate was not critical, but if it was too high crystals were deposited on the specimen.	Depends on the type of specimen; bones can be left in the soln for 2-3 mon without damage	Not given; a long time probably	7	Surgical tools or knife, metal containers Sodium perborate tetrahydrate, detergent	Museum skeletal specimens	Chapman (1969)

CHEMICAL PRESERVATION (Continued)

SPECIMEN DESCRIPTION	ORIGINAL FORM OF SAMPLE	BRIEF DESCRIPTION OF METHOD	LIMITATIONS OF METHOD	OTHER COMMENTS ABOUT METHOD	ESTIMATED PRESERVATION TIME	SURVIVAL TIME	NO. OF STEPS	EQUIPMENT AND REAGENTS USED	END USE OF SPECIMEN	REFERENCE
321. Feces	Fresh pig feces	Pig fecal samples to be analyzed for carbohydrates, were mixed with eq volumes of water and acetone to which toluene was added as a preservative. Aliquots were centrifuged, and the supernatant was treated with zinc sulfate/barium hydroxide, 1:20, to give a protein-free filtrate. (Although the paper does not say so, it was assumed that the samples were refrigerated until analyzed.)	Marked changes in total reducing sugar conc occurs in stored feces, even if they are frozen, unless the samples are deproteinized shortly after collection.		½ hr	At least 5 days	4-6	Metabolism crates, containers, pipettes, centrifuge Acetone, water, toluene, zinc sulfate/barium hydroxide, 1:20	Anal of carbohydrates	Shearer (1969)
322. Flowers	Fresh flower buds on stems	Flowers of several chrysanthemum cultivars were harvested at the 'tight-bud stage'. The foliage was partially trimmed, and the buds were trimmed to 18 in. in length. The stems were placed in vases filled with water plus a commercial floral preservative. Others were placed in water contg 8-hydroxyquinoline and sucrose. The various preservatives were compared for their effect on flower opening, flower size, and bloom preservation.	Flowers held in 8-hydroxyquinoline or Everbloom were larger than flowers held in other preservatives. They were also fully developed and globular in form after 6 days. Stems were heavier with 8-hydroxyquinoline or Everbloom than they were with other preservatives. Flowers held in 8-hydroxyquinoline plus sucrose soln were still good after 2 wk although foliage showed chlorosis.	The largest flowers with the heaviest stems had the longest vase life.	Few min	2 wk	2-3	Scissors; quart glass jars; constant temp, light, and humidity room (greenhouse) Distd water, 8-hydroxyquinoline, Bloomlife, F. M. Super, Roselife, Everbloom, sucrose	To reduce damage and prolong transportation time for commercial flowers	Marousky (1970)
323. Flowers	Gladiolus spikes 2-6 hr old from picking	Gladiolus spikes were conditioned in water or soln of 1,000, 5000 or 10,000 ppm 8-hydroxyquinoline citrate with or without 4% sucrose for 24 hr in an attempt to find a floral preservative.	Spikes conditioned in 1,000 ppm 8-hydroxyquinoline plus 4% sucrose for 24 hr weighed more after 4 days of vase life than spikes conditioned in 8-hydroxyquinoline alone. The preserved spikes were turgid after 4 days while the spikes conditioned with water only showed wilting. Spikes conditioned in 8-hydroxyquinoline at 110°F weighed more after 4 days of vase life than spikes conditioned at 74°F.		Few min	4 days	3	Scissors or knife, plastic containers, constant light and temp room 8-hydroxyquinoline citrate, 4% sucrose soln, water	Botany specimen or decorative flowers	Marousky (1970a)

CHEMICAL PRESERVATION (Continued)

SPECIMEN DESCRIPTION	ORIGINAL FORM OF SAMPLE	BRIEF DESCRIPTION OF METHOD	LIMITATIONS OF METHOD	OTHER COMMENTS ABOUT METHOD	ESTIMATED PRESERVATION TIME	SURVIVAL TIME	NO. OF STEPS	EQUIPMENT AND REAGENTS USED	END USE OF SPECIMEN	REFERENCE
324. Fluorescein soln	Fluorescein sodium soln contaminated with P. aeruginosa	A 2% fluorescein sodium soln was inoculated with Pseudomonas aeruginosa strain NCTC 8203 to make a standard 'contaminated' mixt. The following compds were tested on the mixt as preservatives: 1) Phenylmercuric nitrate, 0.002%, 2) Phenylethyl alc, 0.6%, 3) Phenylmercuric nitrate, 0.002% plus phenylethyl alc, 0.6%, 4) Phenylmercuric nitrate, 0.002% plus phenylethyl alc, 0.4%, 5) Phenylmercuric nitrate, 0.002% plus phenylethyl alc, 0.2%.	Combinations 3 and 4 achieved sterility within 60 min. All achieved sterility after 180 min.	The effectiveness of phenylethyl alc in combination with another chem antibacterial agent against Pseudomonas aeruginosa was probably related to the permeability of the bacteria to the agent.	Few min	Not given	2	Incubator, containers Sodium fluorescein soln, phenylmercuric nitrate, phenylethyl alc	Sterilization of fluorescein sodium soln	Richards (1969)
325. Heart	Excised heparinized whole rat heart	The effects of perfusion with modified Ringer's soln; cardiac arrest using sodium fluoride, adrenochrome, acetylcholine, potassium chloride, or magnesium sulfate; and resuscitation with perfusion fluid contg magnesium sulfate, glutathione, and norepinephrine on excised rat heart storage and metabolism were studied.	Magnesium sulfate-arrested hearts could be resuscitated after 6 hr of arrest. Hearts arrested 4 hr or longer in all soln functioned poorly.	Survival of organs at body temp can be prolonged by metabolic inhibitors. They are thought to inhibit auto-digestion and prevent enzyme depletion and cell intoxication.	5-10 min	Up to 4 hr	6-7	Constant temp bath, Langendorff column, cannulation needles, beakers Oxygenated modified Ringer's soln with 100 mg% dextrose; heparin; pentobarbital; sodium fluoride, 0.8 mg/ml; adrenochrome, 0.2 mg/ml; glutathione, 0.4 mg/ml; magnesium sulfate; acetylcholine; potassium chloride	Heart transplants	Webb (1966)
326. Heart	Canine and human heart-lung preparations	Canine and human normothermic heart-lung preparations were maintained without a pump oxygenator in a fully perfused, forcefully beating stage outside the body by using the pumping force of the graft itself. A stabilizer bag was used to regulate the blood pressure and the blood vol circulating in the prepn according to the pressure in the ascending aorta. The autoperfusate was heparinized to avoid clotting.	Heart-lung prepn could be preserved up to 27 hr. The av was 11 hr 8 min.	After varying times of anoxia cadaver hearts were resuscitated and preserved for several hr using autoperfusion prepn.		Up to 27 hr; av 11 hr	6-7	Stabilizer bag, surgical app, app for suspending excised organs, electrocardiogram, blood pressure monitor, oxygen saturation monitor, pump oxygenator, respirator 25% glucose soln, insulin, heparin	Heart-lung transplants	Robicsek (1969)

CHEMICAL PRESERVATION (Continued)

SPECIMEN DESCRIPTION	ORIGINAL FORM OF SAMPLE	BRIEF DESCRIPTION OF METHOD	LIMITATIONS OF METHOD	OTHER COMMENTS ABOUT METHOD	ESTIMATED PRESERVATION TIME	SURVIVAL TIME	NO. OF STEPS	EQUIPMENT AND REAGENTS USED	END USE OF SPECIMEN	REFERENCE
327. Heart	Freshly excised human and canine heart and lung removed en bloc	Canine and human heart and lung were removed en bloc. The lung was ventilated with a Harvard respirator. The heart and lung were kept at room temp from 30 min-5 hr and were then connected to a pump oxygenator and resuscitated. After restoration of a forceful beat the heart and lung were disconnected from the pump and converted into an autoperfusing heart-lung prepn. Calcium chloride and isoproterenol were given prior to defibrillation, dexamethasone after resuscitation, and glucose and insulin every hr after the 2nd hr of spontaneous activity.	Anoxic periods exceeding 30 min cut down on survival time of the autoperfusing heart-lung prepn directly proportional to the length of anoxia. However, resuscitation of cadaver hearts up to 180 min after cardiorespiratory arrest is feasible.	Survival time of the heart-lung prepn from functioning donors was much better than that of prepn from cadavers.	Varied with amt of anoxia	Up to 7 hr 10 min, after 30 min anoxia (optimum)	7-8	Travenol Miniprime pump, surgical tools, Harvard respirator Calcium chloride; isoproterenol; dexamethasone; 50% glucose soln; 0.21 I.U. insulin; heparin; heparinized, autologous blood	Heart-lung transplant	Tam (1969)
328. Kidneys	Freshly excised canine kidney	Dog kidneys were excised, cannulated, and flushed with 10 ml isotonic saline contg heparin. Dog blood collected in plastic bags contg heparin was used to perfuse the kidney at 25°C in a special perfusion app (described). Other kidneys were perfused with blood to which either hypernatronic of isotonic sodium chloride and 5% glucose were added, and some were perfused with added creatinine and Locke's soln. Blood and urine flow were monitored.	Using heparinized, oxygenated, autologous whole blood with simple diluents such as physiol saline and Locke's soln, it was possible to maintain urine production up to 7 hr. Blood flow was reduced in the hematocrit range of 30-50, and there was reduction of tubular capacity for water reabsorption.	The average ischemic time was 30 min.	½ hr	7 hr	5-6	Perfusion app (described), surgical tools, cannulas, Fenwal blood bags, calibrated hypodermic syringe Autologous blood, sodium chloride, Locke's soln, creatinine, heparin	Kidney transplants	Couch (1958)

CHEMICAL PRESERVATION (Continued)

SPECIMEN DESCRIPTION	ORIGINAL FORM OF SAMPLE	BRIEF DESCRIPTION OF METHOD	LIMITATIONS OF METHOD	OTHER COMMENTS ABOUT METHOD	ESTIMATED PRESERVATION TIME	SURVIVAL TIME	NO. OF STEPS	EQUIPMENT AND REAGENTS USED	END USE OF SPECIMEN	REFERENCE
329. Kidneys	Whole excised primate and sheep kidney	Sheep and baboon kidneys were removed and immediately cooled to 10°C and perfused with 10% low mol wt dextran in physiol saline contg heparin and procaine. The organs were weighed, cannulated, and placed in a perfusion chamber at 37°C. Perfusate in the chamber contained heparinized blood, balanced heparinized blood, balanced salt soln, White's or Hank's soln, dextran, pencillin, and streptomycin. Addition of low mol wt dextran to the perfusate and a low hematocrit contributed to successful maintenance of the excised kidneys.	Baboon kidneys isolated for 24 hr maintained adequate blood flow and urine production. Reimplanted baboon and sheep kidneys that had previously been isolated 5-7 hr resumed relatively normal functioning following contralateral nephrectomy.		½ hr	5-24 hr; optimum 7 hr	5-6	Perfusion app (described), reciprocating pump, surgical tools 10% low mol wt dextran in saline contg heparin and procaine, blood perfusate contg balanced salt soln, Hank's soln, dextran antibiotics	Transplants	Telander (1964)
330. Kidneys	Freshly excised canine kidney	Dog kidneys were excised, weighed and cannulated. The kidneys were then perfused at room temp against a venous pressure (to prevent intrarenal vascular spasm and collapse) with a variety of perfusates until the venous effluent was clear. The perfused kidneys were autotransplanted and observed. The most effective perfusate was 5% low mol wt dextran in balanced salt soln buffered to pH 7.4 and contg heparin.	Kidneys could be preserved up to 48 hr and still produce a successful autotransplant.	Oligouria occurred during the first 36 hr after transplantation.	<1 hr	Up to 48 hr	5-6	Perfusion app, surgical tools, polythene cannula Heparin, 5% low mol wt dextran, balanced salt soln, tromethamine buffer, heparin	Transplants	Manax (1965)

CHEMICAL PRESERVATION (Continued)

SPECIMEN DESCRIPTION	ORIGINAL FORM OF SAMPLE	BRIEF DESCRIPTION OF METHOD	LIMITATIONS OF METHOD	OTHER COMMENTS ABOUT METHOD	ESTIMATED PRESERVATION TIME	SURVIVAL TIME	NO. OF STEPS	EQUIPMENT AND REAGENTS USED	END USE OF SPECIMEN	REFERENCE
331. Kidneys	Freshly excised canine kidney	Several techniques were investigated in an attempt to provide practical means to prolong storage of dog kidney. The most useful technique involved transplantation of the donor kidney into an intermediary recipient, and after a period of storage, into the final recipient. The effects of this form of storage in the transplant kidney function were investigated.	Dog kidneys could be stored for up to 5 days in an intermediary recipient.	This method could possibly be applied to humans by using primates as the intermediary recipients. By using an intermediary it would be easier to discard poorly functioning organs (such as some cadaver grafts) and not cause the human patient to undergo unnecessary surgery. An animal recipient must be sacrificed for this method, and it does not solve the problems of recipient rejection of the transplanted organ.		Up to 5 days	Not clear	Surgical equipment Cytotoxic agents (not specified)	Transplants	Ackermann (1966a)
332. Kidneys	Freshly excised human cadaver kidney	Cadaver kidneys from acceptable donors were removed 5 min after cardiac arrest and cooled by flushing with cold, normal saline or Ringer's lactate soln. They were installed in a sterile LI-400 preservation app and perfused at 8^{o}-$10^{o}C$ with modified human AB+ or AB- plasma that was filtered on a series of Millipore filters. KCl, mannitol, penicillin, Decadron, and insulin were added to the plasma perfusate.	63 patients received cadaver kidneys preserved up to 50 hr. Typical preservation time was 24-36 hr.	Many details of performing kidney removal, preservation, and transportation to a recipient, and prepn of the cadaver and recipient on a routine basis were included.	< 1 hr	Up to 50 hr	4-6	Surgical tools, LI-400 preservation unit (Life Instrumentation, Ill) and mobile van, sterilizing oven, Millipore filters Normal saline soln, Ringer's lactate soln, Human AB+ or AB- plasma KCl, 25% mannitol, penicillin, Decadron, insulin, phenoxybenzamine, heparin	Routine human kidney transplants	Belzer (1970a)
333. Liver and kidneys	Whole canine cadaver	Circulation of 20 dog cadavers was maintained with the Anstadt EMVA (external mechanical ventricular assistor), and their livers and kidneys were employed as allografts implanted 4-24 hr after donor death.	Both livers and kidneys functioned well for 2 days after transplantation. Gross and structural characteristics of the transplanted organs remained normal.		Few min	Up to 24 hr	1	Anstadt external mechanical ventricular assistor, surgical tools	Liver and kidney transplants	Attai (1969)

CHEMICAL PRESERVATION (Continued)

SPECIMEN DESCRIPTION	ORIGINAL FORM OF SAMPLE	BRIEF DESCRIPTION OF METHOD	LIMITATIONS OF METHOD	OTHER COMMENTS ABOUT METHOD	ESTIMATED PRESERVATION TIME	SURVIVAL TIME	NO. OF STEPS	EQUIPMENT AND REAGENTS USED	END USE OF SPECIMEN	REFERENCE
334. Lungs	Freshly excised baboon lung	Excised baboon lungs were wrapped in saline-soaked sponges to prevent drying, and were maintained at 37°C for up to 3 hr. The lung was then perfused with 500 cc of cold, low mol wt dextran and reimplanted. Counting reimplantation time, total ischemia was more than 4 hr.	Lung reimplantation after 4 hr of ischemia resulted in a 30-day mortality rate of 33%.		Few min	Up to 4 hr	3	Surgical tools, saline-soaked sponges, containers Saline soln, low mol wt dextran soln	Lung transplants	Arnar (1967)
335. Lungs, liver, and kidneys	*In situ* dog lungs and cadaver lungs, liver, and kidneys	Canine and human lungs were preserved and transplanted under a variety of conditions involving organs from cadavers and from living donors. The length of time organs can withstand normothermic ischemia and mechanical ventricular assistance before deterioration was investigated. Emphasis was on short-term *in situ* preservation using various perfusion techniques mostly involving Ringer's soln and heparin.	Dog lungs can withstand up to 4 hr of normothermic ischemia. Cadaver lungs, liver, and kidneys could be preserved more than 6 hr *in situ* using a mechanical ventricular assistor after donor death.	Organs from patients who die while on cardiopulmonary bypass would make good transplant specimens.		4-8 hr	Varies	Surgical app Oxygen, nitrogen, perfusion fluids (mostly Ringer's soln with various additives), heparin	Organ transplants	Vieth (1969)
336. Muscle	Freshly excised rat diaphragm and uterine muscles	A synthetic interstitial cell fluid was formulated contg the following: NaCl, 6.3 gm/l; KCl, 0.26 gm/l; $CaCl_2$, 1.7 ml of 10% soln; $MgSO_4$, 0.17 gm/l; $NaHCO_3$, 2.2 gm/l; NaH_2PO_4, 0.26 gm/l; sodium gluconate, 2.1 gm/l; glucose, 1.0 gm/l; and sucrose, 2.6 gm/l. The soln was tried on rat diaphragm and uterus at 25°C, and comparisons with Ringer's and Kreb's soln are given.	Rat diaphragm resting potentials for synthetic interstitial fluid were much closer to those measured *in vivo* then muscles bathed in Kreb's or similar soln.	Other artificial perfusates or tissue maintenance soln (such as Ringer's soln, Kreb's soln, etc.) were based on the composition of blood plasma. The author felt that interstitial fluid should serve as a more accurate model.	Few min	Not given	3-4	Containers, surgical tools, carbogen bubbler NaCl, KCl, $CaCl_2$, $MgSO_4$, $NaHCO_3$, NaH_2PO_4, sodium gluconate, glucose, sucrose	Interstitial fluid replacement soln	Bretag (1969)

CHEMICAL PRESERVATION (Continued)

SPECIMEN DESCRIPTION	ORIGINAL FORM OF SAMPLE	BRIEF DESCRIPTION OF METHOD	LIMITATIONS OF METHOD	OTHER COMMENTS ABOUT METHOD	ESTIMATED PRESERVATION TIME	SURVIVAL TIME	NO. OF STEPS	EQUIPMENT AND REAGENTS USED	END USE OF SPECIMEN	REFERENCE
337. Muscle	Freshly excised frog sartorius muscle	Frog sartorius muscles were excised under sterile conditions, and incubated in a 250 ml screw-cap flask with incubating medium. Carbon dioxide-oxygen mixt (5:95%) was allowed to flow over the surface of the medium to flush air out of the flask, then the flasks were closed and sealed with paraffin. Flasks were shaken in a constant temp bath at $25^{o}C \pm 0.05^{o}C$ or stored in a constant temp room at $25^{o}C \pm 1.0^{o}C$. The sodium, potassium, and resting potential of the incubated muscles were measured over a period of time.	Frog sartorius muscle could be preserved <u>in vitro</u> at $25^{o}C$ for up to <u>8 days</u> with little change in potassium, sodium, and contractility.		Few min	8 days	5-6	Dissecting tools, Erlenmeyer flasks with screw-caps, Aminco constant temp bath or constant temp room, shaker Incubating medium (GIB) contg 42 ingredients (described), carbon dioxide-oxygen (5:95%), parafilm	Muscle tissue specimens	Ling (1969)
338. Muscle	Fresh chicken heart or skeletal muscle	Pieces of chicken heart or skeletal muscle were placed in a vial contg a 2% soln of 2-phenoxyethanol with or without 0.25 M sucrose. The vials were capped and stored at room temp. Serum albumin, lactate dehydrogenase, and malate dehydrogenase activities were measured after 2 wk storage.	Lactate and malate dehydrogenases and serum albumin were normal after 2 wk although the amt was diminished. Physical, catalytic, and immunological properties of the preserved proteins appeared normal after 2 wk.	This preservative was used successfully by the British Museum for animals and plants after fixation with formaldehyde or Bouin's fluid; but unfixed invertebrates disintegrated in this preservative after a few wk. Therefore it is better suited to selected tissues rather than whole organisms. Proteins resistant to thermal denaturation survived for long periods of time in this preservative.	5 min	2 wk or more	2	15 ml vials with caps, tissue mincer 2% (v/v) 2-phenoxyethanol soln	Biochem taxonomy	Nakanishi (1969)

CHEMICAL PRESERVATION (Continued)

SPECIMEN DESCRIPTION	ORIGINAL FORM OF SAMPLE	BRIEF DESCRIPTION OF METHOD	LIMITATIONS OF METHOD	OTHER COMMENTS ABOUT METHOD	ESTIMATED PRESERVATION TIME	SURVIVAL TIME	NO. OF STEPS	EQUIPMENT AND REAGENTS USED	END USE OF SPECIMEN	REFERENCE
339. Sea pudding	Whole sea pudding immersed in sea water	Propylene phenoxetol was added to sea water which contained the marine invertebrate, <u>Stichopus</u> <u>moebii</u>.	Dissected specimens exposed to propylene phenoxetol were still intact after five days and had begun to ooze only slightly. Specimens stored in sea water alone began to ooze and degenerate within minutes after dissection.	The sea pudding undergoes a process of local degeneration when exposed to trauma such as dissection.	1-5 min	Up to 5 days	1	Dissection tools and dish Sea water, propylene phenoxetol	Dissection	Hill (1966)
340. Seed	Stored corn seed	Ceresan and Coversan, an org mercurial fungicide and a non-mercurial fungicide respectively, were dusted on maize seed as dry seed dressings. The excess was shaken off on newspaper. Panogen, a liq mercurial seed dressing, was sprayed on maize seed. Treated seed was kept in closed containers for 24 hr at room temp or at 18°C. Then the seed was stored for varying lengths of time, and the effects of the fungicides on germination were detd.	None of the fungicides reduced the vigor of the seedlings, or caused distortion, for up to 1 yr of storage. Stored, treated seed was not tested in the field or by the cold test method (Hoppe 1955) because soil-borne pathogens could mask any reduction in germination caused by direct effects of the fungicides.		15 min	Up to 1 yr	2-3	Closed containers, constant temp room Ceresan (2.3% phenyl mercury acetate and 0.4% ethoxy mercury silicate), Coversan (96% tetrachloro-<u>p</u>-benzoquinone), and Panogen (2.2% methyl mercury dicyandiamide)	Seed corn	Smith (1970)
341. Spermatazoa	Fresh rabbit ejaculate	Pooled rabbit semen was washed free of seminal plasma by centrifugation within 30 min of collection. Washed sperm was diluted with either coconut milk extender (CME) or Norman Johnson soln No. 2 (N-J-2) to give a final conc of 10-12x10^6 sperm/cc. 2 cc plastic vials were filled completely with suspended cells, tightly capped, and stored in the dark at room temp.	Rabbit sperm stored in CME at room temp was 72% viable and 64% motile after 6 days. Sperm stored in N-J-2 was 69% viable and 72% motile after 6 days. N-J-2 was a better diluent for storing rabbit sperm at room temp because more cells remained motile, and a larger % of cells could be reactivated by resuspension.	This method did not increase acrosome cap loss appreciably.	20 min	6 days	4	Artificial vagina, centrifuge, flasks, 2 cc plastic vials, caps, preservative Coconut Milk Extender (CME) medium, Norman Johnson soln No. 2 (N-J-2) medium, citrate buffer	Study of senescence in living cells	Gulyas (1966)

CHEMICAL PRESERVATION (Continued)

SPECIMEN DESCRIPTION	ORIGINAL FORM OF SAMPLE	BRIEF DESCRIPTION OF METHOD	LIMITATIONS OF METHOD	OTHER COMMENTS ABOUT METHOD	ESTIMATED PRESERVATION TIME	SURVIVAL TIME	NO. OF STEPS	EQUIPMENT AND REAGENTS USED	END USE OF SPECIMEN	REFERENCE
342. Spores	Fungal spores in static malt broth cultures	The effects of preservatives on the swelling characteristics of several types of spores were examined using the Coulter Counter. Penicillium spinulosum was chosen as a test organism for assessing preservative efficiency because it began swelling earliest and had the most rapid swelling rate. The preservatives propylhydroxybenzoate, Phenonip (Nipa labs), and benzoic acid were evaluated by this method.	Propylhydroxybenzoate and Phenonip decreased the swelling rate of germinating fungal spores, while benzoic acid increased the time before swelling began.	This method is useful for screening chemical preservatives.			2-3	Coulter Counter Model B, incubator, calipers Culture medium, benzoic acid, propylhydroxybenzoate, Phenonip (Nipa Labs)	Screening method for chemical preservatives	McCafferty (1970)
343. Tendon	Freshly excised rabbit calf tendon	Rabbit tendon homotransplant tissue was preserved in one of the following solutions: 1) AGDM contg aminocrovin, 85.0; glycerine, 15.0; diprazin, 1.0; micerin, 300,00 IU, or 2) RGDM in which Ringer-Locke soln was used instead of aminocrovin. The samples were maintained at 2° and 4°C for 30-60 days. Phys properties and histochem properties were observed, and transplants were made to test the effectiveness of the storage.	AGDM preserved tendons for 30 days and RGDM for 60 days successfully. Sterility of the solutions was maintained for 18 mon.	After 6 mon the transplant was entirely replaced by new live cells which grew into the transplant without incapsulation or scar formation.	Few min	30-60 days	3	Surgical tools, refrigerator Aminocrovin, glycerine, diprazin, micerin, Ringer-Locke soln	Calf-tendon transplant	Beringer (1969)
344. Tissue cultures	WI-38, HEp-2, and primary bovine kidney cell cultures	Polyvinyl trays used in antiviral screening were sterilized with 100% ethylene oxide for 5 hr after 48 hr of prehumidification, or by 12% ethylene oxide plus 88% Freon for 1, 3, 5, or 18 hr. The sterilization of the trays left a residue of ethylene oxide that was toxic to the primary bovine kidney cells usually grown on them. Various types of aeration were tried to remove the toxic residue.	Exposure to 12% ethylene oxide plus 88% Freon for one hr followed by a 7-day aeration period at 37°C was sufficient to sterilize the trays and eliminate toxicity to cell monolayers grown on the trays. Sterilization with 100% ethylene oxide required 14 days aeration at 37°C to eliminate cytotoxicity.	Polyvinyl trays used for antiviral screening were subject to seasonal outbreaks of bacterial and mycotic contamination of the tissue culture cells. Ethylene oxide was an effective sterilant but it left a toxic residue.	1 hr		6-8	Linbro FB-54 clear polyvinyl trays, dishwasher, detergent, polyethylene bags, paper bags, stapler, cotton, heat sealer, Amer ETO sterilizer, vacuum oven Ethylene oxide, Freon, growth medium	Antiviral screening	Rueter (1969)

CHEMICAL PRESERVATION (Continued)

SPECIMEN DESCRIPTION	ORIGINAL FORM OF SAMPLE	BRIEF DESCRIPTION OF METHOD	LIMITATIONS OF METHOD	OTHER COMMENTS ABOUT METHOD	ESTIMATED PRESERVATION TIME	SURVIVAL TIME	NO. OF STEPS	EQUIPMENT AND REAGENTS USED	END USE OF SPECIMEN	REFERENCE
345. Tympanic membrane	Tympanic membrane-ossicular chain tissues removed from a human cadaver	Homograft tympanic membrane-ossicular chain transplants, obtained through routine autopsy, were stored in a soln of Zephiran chloride and Tis-U-Sol. (No proportions were given).	Homografts produced effective transplants after storage. Length of stored time was not given.	Very little detail about the storage method was given.	Few min	Not given	2	Surgical tools, containers Zephiran chloride, Tis-U-Sol	Transplants	House (1969)
346. Urine	Fresh human urine	Toluene or hydrochloric acid was recommended for preservation of urine.	No detail given		Few min	Not given	2-3	Flasks Toluene, HCl	Urinalysis	Brandt (1924)
347. Urine,	Fresh human urine	Phenylpyruvic acid in urine was preserved for long periods by adding a little chloroform to the samples and acidifying to pH 4 with dilute HCl.	Samples could be stored for 'long periods' using this method.	Phenylpyruvic acid is found in the urine of certain mental patients.	Few min	'long periods'	2-3	Flasks Chloroform, HCl	Anal of phenylpyruvic acid	Rhein (1936)
348. Urine	Fresh urine	10 mg/l of ethylmercuric thiosalicylate and phenylmercuric borate were used to preserve urine samples at room temp.	The method did not interfere with standard anal of urine, nor did the reagents attack aluminum containers.		Few min	Not given	1-2	Containers, pipettes Ethylmercuric thiosalicylate, phenylmercuric borate	Standard urine anal	Fabiani (1951)
349. Urine	Fresh human urine	Urine collected for anal of corticosteroids was preserved from bacterial contamination by addition of chloroform followed by refrigeration. The corticosteroids were maintained by adding 5 gm of a mixt of potassium dihydrogen phosphate and disodium hydrogen phosphate, 2:1, to each liter of urine. This kept the pH at approx 6.5.	Urine samples were stable for 48 hr if buffered to pH 6.5. Refrigeration and addition of chloroform were recommended to cut down bacterial contamination.		Few min	48 hr	3	Containers, refrigerator Chloroform; mixt of potassium dihydrogen phosphate and disodium hydrogen phosphate, 2:1	Corticosteroid anal	Tompsett (1955)

CHEMICAL PRESERVATION (Continued)

SPECIMEN DESCRIPTION	ORIGINAL FORM OF SAMPLE	BRIEF DESCRIPTION OF METHOD	LIMITATIONS OF METHOD	OTHER COMMENTS ABOUT METHOD	ESTIMATED PRESERVATION TIME	SURVIVAL TIME	NO. OF STEPS	EQUIPMENT AND REAGENTS USED	END USE OF SPECIMEN	REFERENCE
350. Urine	Fresh or 24 hr human urine collection	5 ml of a 10% thymol soln in isopropanol/24 hr urine sample kept urine from deteriorating for 7 days.	Urine samples were good for 7 days at room temp when preserved by this method. The method was compatible with most routine urine anal.	Thymol-isopropanol preservation of urine causes interference with the Hay's test for bile salts and the Zimmermann reaction for 17-ketosteriods.	Few min	Up to 7 days at room temp	1	Flasks or containers	Urinalysis	Naftalin (1958)
351. Urine	Midstream fresh human urine sample	A clean, midstream, human urine specimen was obtained and immediately diluted 1:2 with formalin soln (37% formaldehyde soln, 100 ml; sodium acetate, 25 gm; distd water, 900 ml) which acts as a preservative and fixative. Within 5 days after collection the preserved specimen was filtered through a filter membrane which had been moistened with physiol saline. The filter was rinsed with saline, and the vacuum was broken just before the rinse was complete to avoid cell distortion. Formalin-preserved specimens required no further fixation, but if fresh cells were used, they were fixed with alc. After fixation the cells were stained with Shorr's stain and counted for casts and cells.	89% of samples fixed with formalin were suitable for quantitative examination after 7 days at 37°C.	Precipitate formation was the principal drawback of this method.	Approx ½ hr	7 days	6-8	Side arm flask and filter, 1.2 μ Millipore filters, vacuum source microscope slides, cover slips, micrometer Formalin soln, Shorr stain, ethanol, isopropyl alc, xylene, and mounting fluid	Quantitative anal of cells and casts in urine	Teitel (1964)
352. Urine	24 hr urine sample	24 hr urine samples were collected from suspected cases of phaeochromocytoma during a hypertensive phase. Total vol was measured, and a 50 ml sample was taken. 150 mg of ascorbic acid in pure form (either powd or from an ampule) was added to the urine, and the pH was maintained at 5.5. All patients were free from hypotensive drugs or barbiturates for at least 2 days prior to the urine collection.	Not given	Vitamin C tablets should not be used because they have an alk base which may oxidize the pressor amines. The paper did not state that 24 hr urine samples were refrigerated, but this was probably the case.	5-10 min	Not given	4	Urine collection containers Pure ascorbic acid, powd or liq	Assay of catecholamines in urine	Fernando (1969)

CHEMICAL PRESERVATION (Concluded)

SPECIMEN DESCRIPTION	ORIGINAL FORM OF SAMPLE	BRIEF DESCRIPTION OF METHOD	LIMITATIONS OF METHOD	OTHER COMMENTS ABOUT METHOD	ESTIMATED PRESERVATION TIME	SURVIVAL TIME	NO. OF STEPS	EQUIPMENT AND REAGENTS USED	END USE OF SPECIMEN	REFERENCE
353. Vaccines, tissue grafts or blood plasma	Fresh plasma, cadaver tissue, or viral cultures	0.45% beta-propiolactone was used, with or without UV irradiation, to sterilize human blood plasma. The sterilant was also used on arteries, bones, kidney, liver, and cartilage for transplants, and to produce inactivated virus vaccines.	Beta-propiolactone caused a rapid and irreversible sterilizing action. It produced minimum alteration of proteins thus avoiding final neutralization, and it also produced relatively non-toxic end products. The sterilant rapidly penetrated tissue, and inactivated viruses completely and irreversibly in 10-15 min at 37°C.		Few min	Plasma keeps for 'long periods'		Beta-propiolactone	Sterilization of plasma and tissue, and viral antigen production	Hartman (1957)
354. Viruses	Allantoic fluid infected with influenza virus	Influenza-infected allantoic fluid contg penicillin and streptomycin was added to sterile precipitation tubes and sealed with hot solid paraffin. The tubes were stored in the dark at 26°-30°C.	The influenza isolates stored by this method maintained 80-100% viability for 25-35 days at room temp.	Tubes of influenza isolates prepd in this manner were suitable for mailing or shipping. The method may be applicable to other viruses.	5 min	25-35 days at room temp	2	Hypodermic syringe, precipitation tubes, sterilizer Paraffin	Simple virus storage for shipping	Gan (1970)

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DRYING AND HEAT STERILIZATION

SPECIMEN DESCRIPTION	ORIGINAL FORM OF SAMPLE	BRIEF DESCRIPTION OF METHOD	LIMITATIONS OF METHOD	OTHER COMMENTS ABOUT METHOD	ESTIMATED PRESERVATION TIME	SURVIVAL TIME	NO. OF STEPS	EQUIPMENT AND REAGENTS USED	END USE OF SPECIMEN	REFERENCE
355. Amoeba	Aq or ethanol suspension of amoeba or other samples	Small polystyrene cubes (0.6-0.9 mm per edge) were cut with a razor blade, weighed, and impaled on a glass needle. The sponage was dipped into 5-10 µl of a suspension of amoeba collected in the tip of a microcone. Both sponge and microcone were under vacuum to remove air and allow the sponge to fill. After filling the sponge was allowed to dry. The whole process was repeated 6-7 times, and before the last drying the needle was removed to prevent it from sticking to the sponage.	Not given, since emphasis here was on technique for weighing dispersed samples on a quartz-fiber microbalance. However, the sponge held six to seven times its own wt of specimen and could be a useful preservation technique. Also the wt of the loaded sponge did not change after repeated handling.	Polystyrene dissolves in most org solvents but is impervious to ethanol, water and most aq reagents. Sponges can be heated to 65°C for drying specimens, but soften and collapse above this temp.	Depends on how many times sponge is filled and dried	Not given; however, samples dried in this manner could presumably be stored for sometime, if kept dry	4-5	Polystyrene sponge cubes, glass needle, ethanol or aq suspension of sample, razor blade, drying oven, container under vacuum Ethanol	Weighing dispersed samples on a quartz-fiber fishpole microbalance	Thomas (1959)
356. Plasmagel	Sterile plasmagel samples stored in hermetically sealed vials	Modified fluid gelatin soln (Plasmagel) was stored in hermetically sealed 125 ml glass vials. The vials were exposed to temp between -5°C and 45°C and to ultrasonic vibration to simulate conditions that might exist during long-term storage.	Ultrasonic vibration had a negligible influence on Plasmagel whereas elevated temp led to slight changes of fluidity and acidity. 20°C was the optimum storage temp for long term storage.	Plasmagel would be needed in great amt in the case of a national diaster. This paper attempted to ascertain how long its shelf life was under normal storage conditions.		> 70 days	3	Sterilizer, glass vials	Blood substitute	Thiercelin (1969)
357. Feces	Fresh Pig feces	Pig feces were collected and analyzed for various nitrogen factors before and after drying. All feces were collected immediately after being passed, put in a capped glass jar, and stored in the refrigerator. Half of a composite 2-day sample was dried on porcelain trays for 24 hr at 60°C. Dry matter, total nitrogen, and true protein content were detd on both fresh and dried samples. Nonprotein nitrogen was detd in protein-free filtrates, and ammonia nitrogen was detd in water exts. Chromium sesquioxide was used as an indicator, and its level in fresh samples was detd by ashing and spectrophotometry.	The % loss in the total-, protein-, and ammonia-nitrogen content in dried feces as compared to fresh feces was 7.9, 10.8, and 38.1% respectively. The nonprotein nitrogen content increased after drying.	The protein nitrogen fraction of dried pig feces probably decreased because of bacteria and proteolytic enzymes that are present in feces.	24 hr	Not given	3-5	Glass jars, refrigerator, drying oven, porcelain tray, centrifuge filter funnels, crucibles Chromium sesquioxide, sodium peroxide, sodium carbonate	Anal of fecal nitrogen fractions	Ziolecka (1968)

DRYING AND HEAT STERILIZATION (Continued)

SPECIMEN DESCRIPTION	ORIGINAL FORM OF SAMPLE	BRIEF DESCRIPTION OF METHOD	LIMITATIONS OF METHOD	OTHER COMMENTS ABOUT METHOD	ESTIMATED PRESERVATION TIME	SURVIVAL TIME	NO. OF STEPS	EQUIPMENT AND REAGENTS USED	END USE OF SPECIMEN	REFERENCE
358. Insect larva	'Dry' Polypedilum larvae (relative humidity less than 10%)	Larvae of Polypedilum vanderplanki, Hint. were exposed to various regimens of dehydration and temp extremes. They were frozen in liq helium or liq air; they were exposed to temp ranging from 102°-200°C; they were immersed in abs alc or pure glycerol; and they were repeatedly dehydrated and rehydrated under various conditions of initial moisture content. The larvae were considered partially recovered if the pharyngeal or esophageal beat returned, and a response to stimuli was obtained The larvae were considered fully recovered when they were able to metamorphose.	Freeze-thawed larvae metamorphosed, if they were dry when frozen. Some metamorphosed after exposure to 102°-104°C for 1 min when dry, and some recovered temporarily after exposure to 106°C for 3 hr or 200°C for 5 min. Larvae immersed in glycerol for 1, 3, or 7 days metamorphosed, and one metamorphosed after immersion in abs alc for 24 hr. The larvae can stand repeated dehydration and partial dehydration (up to 10x), and some larvae recovered temporarily after being stored dry for 10 yr.	The capacity of 'dry' inactive larvae to withstand prolonged periods of storage or extremes of heat and cold is greater the drier they are.	Varied with expt	Up to 10 yr in dry state	Varied	Petri dishes, filter paper, incubator, glass tubes with 5-8 mm of mud at the bottom, drying oven, dessicator, wash bottle, watch glasses, glass tubes in a metal container Tap water, $CaCl_2$, silica gel, phosphorus pentoxide, liq air, liq helium	Studies of adaptation of animals to extreme temp and moisture conditions	Hinton (1960)
359. Microorganisms	Freshly cultured bacteria and coliphage T_2	Pseudomonas fluorescens was grown on Brain Heart Infusion aerated at 30° for 20 hrs. Coliphage T_2 lysate was prepared from E. coli B. The bacteria were centrifuged, resuspended in distilled water, and mixed with an equal vol of T_2 lysate. Aliquots were diluted with a mixt of distd water, mannitol and sucrose. Samples of these suspensions were placed in ampules and dried in a vacuum dryer at -20°, 0°, and 20°C.	The drying temp had no significant effect on the av number of Pseudomonas fluorescens that survived drying, but coliphage T_2 av survival increased as the temp rose from -20° to 20°C. Temp effects were also dependent on the conc of solutes present.		Several hr	Not given	5-6	Shaker, flasks, filters refrigerator, centrifuge, ampules, vacuum dryer, 40-gauge thermocouples, recording potentiometer Brain Heart Infusion, distd water, mannitol, sucrose	Maintenance of bacterial strains	Leach (1959)
360. Microorganisms	Microorganisms suspended in an appropriate culture medium	The specimens were grown on a specified, aerated medium and collected by centrifugation. The collected cells were resuspended in skim milk precooled to 0°C, and added dropwise to a chilled tube half full of sterile, anhydrous silica gel. The tubes were placed in a dessicator for 1 wk, then each tube was sealed and stored in a sealed jar contg silica gel at 2°-4°C.	The survival rate varied from 13-111 wk depending on the species. Viable Thiobacillus theoparus, Chlamodomonas eugametos, and Euglena gracilis could not be recovered by this method.		15 min	13-111 wk	6	Centrifuge, plastic centrifuge tubes, pyrex glass tubes, cotton-wool stoppers, autoclave, dessicator, refrigerator Reconstituted skim milk, 15% w/v (Bonlac); milk, 7.5%, w/v; anhydrous silica gel; Parafilm	Maintenance of microbial strains	Grivell (1969)

DRYING AND HEAT STERILIZATION (Continued)

SPECIMEN DESCRIPTION	ORIGINAL FORM OF SAMPLE	BRIEF DESCRIPTION OF METHOD	LIMITATIONS OF METHOD	OTHER COMMENTS ABOUT METHOD	ESTIMATED PRESERVATION TIME	SURVIVAL TIME	NO. OF STEPS	EQUIPMENT AND REAGENTS USED	END USE OF SPECIMEN	REFERENCE
361. Mosquitoes	Whole adult or larval insect	Adult mosquitoes were anesthetized with ether, then placed on 2 layers of filter paper surrounded by a ring of cotton in a Petri dish. The cotton was satd with acetone, and the dish was covered for 3 hr. The specimens were then dried under an electric light bulb to remove the excess acetone. A similar technique was used for larvae, except the larvae were immersed in acetone. This technique preserved the insects sufficiently for taxonomic studies.	Adult insects were not shriveled, and the scales and genitalia were intact; however, the eyes lost their natural color. Larvae had hairs intact and did not lose body parts easily. Specimens were durable and could be pin-mounted.	The technique works for several other kinds of insects and is particularly suitable for field work.	3-3½ hr	Not given; probably more than 6 mon	3-4	Petri dish and cover, cotton, filter paper, light bulb, dropper Ether, acetone	Taxonomic studies	Truman (1968)
362. Organic compounds, Volatile	Volatile org compds	Sterilization of solutions of volatile org compds was carried out by placing 5 ml of the volatile compd into glass ampules immersed in ice water. The ampules were heat-sealed 5 cm above the soln level and autoclaved. Recoveries were checked by gas chromatography and a flame ionization detector.	Recovery from autoclaved culture tubes was 90% or greater at all times, while recovery from sealed glass ampules was 96-100% except in the cases of ethyl acetate and ethanol which were in the 90-94% recovery range. No convincing explanation for this loss was given.	Volatile org compds often need to be added to microbiol or biol preparations and they must be sterile to avoid contaminating the specimen to which they are added.	½ hr	Not given	3	Ampules, heat-sealer, autoclave	Production of sterile org volatile compd or addition to biol preparations	Bills (1967)
363. Protein	Protein soln	Rods of dried polymeric hydrophilic gel were dropped into protein soln to remove water.	Protein dried by this technique appeared to be unaltered in structure or properties.	This method was much quicker than freeze-drying or dialysis, and a constant ionic strength was maintained.	10-20 min	Not given	3	Silicon-coated glass tubes, test tubes, centrifuge, glass dish, cold room, dessicator Rods of hydrophilic gel, phosphorus pentoxide	Studies of intact proteins	Curtain (1964)

DRYING AND HEAT STERILIZATION (Continued)

SPECIMEN DESCRIPTION	ORIGINAL FORM OF SAMPLE	BRIEF DESCRIPTION OF METHOD	LIMITATIONS OF METHOD	OTHER COMMENTS ABOUT METHOD	ESTIMATED PRESERVATION TIME	SURVIVAL TIME	NO. OF STEPS	EQUIPMENT AND REAGENTS USED	END USE OF SPECIMEN	REFERENCE
364. Protein	Heat-coagulated leaf protein	Heat-coagulated leaf protein that had been filtered through drill stockings, was pressed at 75 kg/cm^2 for 8 hr to give a cake contg 65-75% moisture. The wet cake was crumbled into 0.5-2 mm particles and air dried in a 40°C warm air-flow to a moisture level at which the particles no longer formed a paste when compacted. The particles were finely ground with a mortar and pestle or end runner mill to a fine green powd. This powd could be further dried to the desired extent without change in appearance.	Samples dried to 6.9% moisture were stored for 6 mon and were predicted to be good for more than 2 yr.	The nutritional value of the protein was damaged if the temp exceeded 82°C; oxidation of unsatd lipids was the main storage problem. Enzyme digestibility of the air-dried protein was comparable to freeze-dried leaf protein.	Approx. 12 hr	6 mon	6-7	Drill stockings, press, food cutter or grater, end runner mill, drying oven or tumble dryer	Food protein	Arkcoll (1971)
365. Protozoa	Protozoan suspension	Culture medium contg *Strigomonas oncopelti* was dropped onto freeze-dried peptone plugs in ampules and dried in a supercooled state at -9°C.	The organisms exhibited good survival as long as drying was carried out from a liq film and not from the frozen state.	Protective additives such as glycerol cannot be used for freeze-drying because, being nonvolatile, they concentrate to toxic levels. Different organisms have different requirements for residual moisture, and the removal of this residual moisture kills the organism.	5 min	Not given	2	High vacuum, ampules, freeze-drying app Peptone plugs	Maintenance of organism line	Greaves (1963)
366. Spores	Dried bacterial spores	Dried *Bacillus subtilis* spores were tested for resistance to dry-heat thermal destruction in a specially-designed dry-heat oven. Both air and nitrogen gases were passed over the organisms. Higher flow rate of gas was believed to cause greater dehydration of the spores, and moisture loss was a major factor in dry-heat thermal destruction of bacterial spores. Dextrose-tryptone-starch-agar medium was used as the protective agent.	90% of the spores were destroyed in 43 min at 140°C in still air. Tables of other conditions were given.		Not clear	< 43 min at 140°C	3-4	Special drying oven, Petri dishes, test tubes, shaker Dextrose-tryptone-starch-agar medium, compressed air, nitrogen	Heat sterilization studies	Fox (1968)

DRYING AND HEAT STERILIZATION (Concluded)

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367. Urine	Fresh human urine	Absorbent materials such as filter paper, woven glass cloth, or fibrous glass were treated with a preservative such as 2.5-4% sodium fluoride, mercuric chloride, merthiolate, or other mercury compds. 8-10 drops of urine were collected on these absorbents and allowed to dry. The samples were then stored at room temp until analyzed.	The samples may be kept for indefinite periods and eluted with suitable solvents prior to anal. For quantitative inorg tests no preservative was needed.		Few min	Indefinite time	1-2	Filter paper disks, fiberglass cloth, glass fibers 2.5-4% sodium fluoride, mercuric chloride, merthiolate, mercury compds	Urinalysis	Drey (1953)

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FIXATION AND EMBEDDING

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368. Blood cells, Red	Sensitized sheep red blood cells	A method for preparing stable, antigen-coated red blood cells by fixation with neutralized glutaraldehyde is outlined.	Fixed red blood cells remained stable up to 6 mon at 4°C.			6 mon	>10	Incubator, ice bath, Bronwill Biosonic II sonicator, test tubes, pipettes, V plates (Cooke Eng Co) Culture media, physiol saline soln, phosphate-buffered saline, tannic acid, $CaCO_3$, 1% glutaraldehyde in saline, sensitized sheep red blood cells, sodium azide, sodium merthiolate	Prepn of stable antigen-coated red blood cells	Neimark (1968)
369. Brain	Whole excised brain	Histologic sections of whole brain were prepared as follows: The brain was immersed in 10% buffered neutral formalin for 14 days, then sliced no more than 1.5 cm thick. The slices were washed with water, submerged, and dehydrated with constant agitation in a series of alc and chloroform for 3 days. The dehydrated specimen was impregnated with liq Paraplast in a vacuum oven at 60°C and was ready for storage, or sectioning with a microtome and staining.	Histologic sections of whole brain can be prepared in a little over 1 wk instead of several wk or mon. The sections lost about 10% of their original vol.		5 days	Not given	6-8	Bausch and Lomb sliding microtome, water bath, glass slides, 1000 ml rectangular dishes, cover glasses, mag stirrer, metal pan, cover dish, vacuum oven, warming table Chloroform, alc (several conc), gelatin, Harris' hematoxylin stain, alcoholic eosin stain, Paraplast	Brain studies	Prophet (1969)

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370. Esophageal varices	Freshly excised cadaver esophagus	Before removal from the body the esophagus was tied off at both ends to prevent blood loss. The esophagus was then removed, untied, opened, and placed in 10% formaldehyde soln. After 24 hr the mucosal layer was stripped from the muscularis and the esophagus was dehydrated in three changes of 50, 95, and 100% alc with 1 hr between changes. The flat specimen was dried between towels and submerged in benzene for clearing for 30-60 min. Then a thin layer of Permount was poured over the specimen in the inverted top of a Petri dish. The bottom was used as a press to force out air bubbles, and the two dishes with the specimen between were clamped and placed in an incubator to dry. 24 hr later more Permount was added between the dishes to force out any air bubbles left. The sample was incubated for 5-7 days, and an additional top was put on as a cover and taped with transparent tape.	Esophageal varices preserved in this manner maintained their color and transparency for up to 18 mon.	The same method can be used to demonstrate enlargement of the vascular channels of the diaphragm, particularly in cirrhosis.	1 wk	> 18 mon	> 10	Surgical tools, ties, containers, paper towels, tray, Petri dishes, C-clamps, incubator, micropipettes, transparent tape 10% formaldehyde soln, 50, 95, and 100% alc soln, benzene or clearing soln, Permount	Demonstration of vascularization	Chomet (1969)
371. Fish, mollusks, and other biol specimens	Whole organisms or organs	A 10% soln of acetic anhydride or anhydrous monomer was used to acylate biogenic amines in water-alc dried biol specimens embedded in acrylic or polyester casting resins. These amines caused delayed formation of bubbles, especially in embedded specimens of mollusks, fish, and isolated organs of higher animals. Acylation took from 1-3 days before embedding.	This technique eliminated bubbles and blurring around the specimen. Specimens should be preserved indefinitely by this method.	Acid chlorides were not recommended.	3-4 days	Indefinitely	4	Mold, containers for alc drying and acylation Acrylic or polyester casting resin, acetic anhydride (or other anhydride), alc	Production of permanently embedded biol specimens	Hofer (1964)

FIXATION AND EMBEDDING (Continued)

SPECIMEN DESCRIPTION	ORIGINAL FORM OF SAMPLE	BRIEF DESCRIPTION OF METHOD	LIMITATIONS OF METHOD	OTHER COMMENTS ABOUT METHOD	ESTIMATED PRESERVATION TIME	SURVIVAL TIME	NO. OF STEPS	EQUIPMENT AND REAGENTS USED	END USE OF SPECIMEN	REFERENCE
372. Kidneys	Fresh sliced rat kidneys	Cortical slices were fixed in 10% neutral, phosphate-buffered formalin; embedded in paraffin; and stained with hematoxylin and eosin.	The embedded samples cannot easily be used for further anal.		½ hr	Indefinite if kept away from heat	5	Petri dishes, tissue slicer Paraffin, buffer, stains	Study of amino acid transport by kidneys	Lowenstein (1968)
373. Kidneys	Fresh sliced rat kidney	Cortical slices were fixed in 3% phosphate-buffered glutaraldehyde, washed in phosphate-buffered saline and post-fixed in 1% phosphate-buffered osmic acid. Samples were dehydrated in graded ethanol, embedded in epoxy resin, and stained with lead citrate and uranyl acetate.	Samples cannot easily be used for further anal.		1 hr	Indefinite	7	Epoxy resin, several containers for dehydrating, tissue slicer Buffer, glutaraldehyde, buffered saline, osmic acid, ethanol, epoxy resin, lead citrate, uranyl acetate	Study of amino acid transport by kidneys	Lowenstein (1968)
374. Liver	Blocks of fresh rat liver tissue	The glycogen content of fresh rat liver was preserved in tissue blocks by several common fixation methods including freezing on carbon dioxide, and treatment with Lavdowsky's fluid or Rossman's fluid. Fixed tissues were lyophilized or embedded in paraffin, and some were also rehydrated. The effect of fixation or fixation plus rehydration on glycogen content was studied.	Freeze-drying, Lavdowsky's fixation, and Rossman's fixation gave quantitative preservation of glycogen. Formalin and 80% ethanol did not. Glycogen preservation in rehydrated tissue sections was variable.	Rehydration of fixed sections destroyed the glycogen. No explanation was given.	5 min-24 hr depending on method	Not given	Depends on method	Microtome, stain dishes Paraffin, formalin, ethanol, Lavdowsky's fluid, Rossman's fluid, dry ice, 30% potassium hydroxide	Preservation of histochem sections with glycogen intact	Swigart (1960)
375. Protozoa	Whole protozoa	A method was described for maintaining the natural shape and size of protozoa for viewing with an electron microscope. Specimens were washed, then fixed with Parducz' fixative contg osmium tetroxide and aq mercuric chloride. Next they were washed again and added as microdrops to liq nitrogen. The frozen drops were freeze-dried and then fixed with gold and palladium vapor under vacuum until examination.	Specimens maintained a great deal of detail and were not misshapened or distorted. They can be reviewed under the microscope several times.	The method can be used for other small vol biol materials.	12-13 hr	Not given	10-11	Centrifuge, micropipette aluminum containers, aluminum discs aluminum planchet, Pearse tissue dryer Distilled H_2O, inorg salt soln, Parducz' fixative, liq nitrogen	Electron microscopy	Small (1969)

FIXATION AND EMBEDDING (Continued)

SPECIMEN DESCRIPTION	ORIGINAL FORM OF SAMPLE	BRIEF DESCRIPTION OF METHOD	LIMITATIONS OF METHOD	OTHER COMMENTS ABOUT METHOD	ESTIMATED PRESERVATION TIME	SURVIVAL TIME	NO. OF STEPS	EQUIPMENT AND REAGENTS USED	END USE OF SPECIMEN	REFERENCE
376. Reticulin fibers		A method for the demonstration of reticulin in paraffin sections was described, which reduced the risk of silver precipitate formation and the impregnation of nuclei by silver.	Not given		½-1 hr	Not given	> 10	Microtome, stain dishes Potassium permanganate, 5% oxalic acid soln, distd water, 5% ammonium ferric sulfate soln, ammoniacal silver soln, formaldehyde soln, 0.2% gold chloride, 5% sodium thiosulfate, neutral red stain, ethanol	Demonstration of reticulin by staining	Chadwin (1969)
377. Salt gland	Freshly extd duckling salt gland or *Fundulus grandis* gill filament	Razor-sliced salt-gland tissue slices weighing 20-40 mg were covered with 5% agar, cooled 5 min at 4°C, and chopped on a tissue chopper to 100-200 μ. Tissue sections were fixed at 4°C with 2 or 3% formaldehyde prepared from paraformaldehyde. Fixative was buffered with 0.1 M cacodylate to pH 7.2. Sections were rinsed in buffer, then homogenized in cold 0.25 M sucrose, and finally incubated in the Wachstein-Meisel medium. The same technique was applied to teleost gill filaments.	Fixation of avian salt-gland tissue with 2 or 3% formaldehyde resulted in a loss of only 30% Na-K-ATPase activity and 65% Mg-ATPase activity after 60-90 min. Whereas fixation with glutaraldehyde completely inhibited Na-K-ATPase and reduced Mg-ATPase by 85%. Similar results were observed with teleost gill filaments.	The method was not tissue or species specific, and the fine structure of the tissue was preserved. Commercial formalin was not suitable due to the methanol preservative used in it.	1 hr	60-90 min	7-8	Razor blade, Smith-Farquhar tissue chopper, flasks, incubator Formaldehyde, cacodylate buffer, sucrose soln	Anal of Na-K-ATPase and Mg-ATPase	Ernst (1970)
378. Spermatozoa	Fresh human ejaculate	Sperm were ejaculated into a buffered picric acid-formaldehyde fixative (a modified Bouin fluid), the mixt was centrifuged for 10 min, and the supernatant discarded. The remaining pellet of spermatozoa was washed in phosphate buffer, post-fixed with osmium tetroxide, and rapidly dehydrated. The pellet was reduced to small fragments and embedded in Epon 812.	Spermatozoa fixed in this manner showed intact plasma membranes, well preserved acrosomes, small nuclear vacuoles, well defined flagella, and mitochondria of uniform density.	In this method some inorg crystals form.	1 hr	Not given	8	Centrifuge, collection vials, hot plate, cylinder, filter funnel Fixative contg paraformaldehyde, picric acid, and sodium hydroxide (shelf life 12 mon); Epon 812; osmium tetroxide; phosphate buffer	Electron microscopy	Stefanini (1967)

FIXATION AND EMBEDDING (Concluded)

SPECIMEN DESCRIPTION	ORIGINAL FORM OF SAMPLE	BRIEF DESCRIPTION OF METHOD	LIMITATIONS OF METHOD	OTHER COMMENTS ABOUT METHOD	ESTIMATED PRESERVATION TIME	SURVIVAL TIME	NO. OF STEPS	EQUIPMENT AND REAGENTS USED	END USE OF SPECIMEN	REFERENCE
379. Tissue culture cells	Fresh liver tissue, cultured Chinese hamster cells, and cultured *Escherichia coli*	A method was described for extracting DNA from tissues and cells fixed with a variety of common fixatives such as might be available on a field trip. Cells were removed from the culture flask with 0.1% Pronase soln. The cell suspension was centrifuged and the pellet was dispersed in an appropriate fixative such as 95% ethanol; abs methanol; 1 abs methanol: 9 ethanol; 5 abs methanol:5 ethanol; 3 methanol:1 glacial acetic acid; 2-propanol; acetone; or 10% formalin in phosphate buffer, for 4 to 66 days. After fixation, cells were washed and the DNA was extd by the method outlined in the paper.	The purity of DNA prepared from fixed mammalian material compares favorably with DNA from fresh or frozen samples except for samples from formalin fixed tissue. Bacterial DNA after fixation had lower absorbance ratios than controls. 2-propanol was the best and most convenient fixative for most materials.		4-66 days	Not given	3-4	Culture flasks, centrifuge, other containers 0.1% Pronase soln, 95% ethanol, abs methanol, glacial acetic acid, 2-propanol, acetone, 10% formalin in phosphate buffer	Isolation and characterization of DNA	Arrighi (1968)

CULTURING

SPECIMEN DESCRIPTION	ORIGINAL FORM OF SAMPLE	BRIEF DESCRIPTION OF METHOD	LIMITATIONS OF METHOD	OTHER COMMENTS ABOUT METHOD	ESTIMATED PRESERVATION TIME	SURVIVAL TIME	NO. OF STEPS	EQUIPMENT AND REAGENTS USED	END USE OF SPECIMEN	REFERENCE
380. Blood cultures	Freshly drawn human blood	Blood samples were introduced into plastic bags contg a sterile medium comprised of 5% aq glucose with Proteose Peptone #3, Tryptose, yeast ext, sodium chloride, and disodium phosphate added. The medium diluted the blood, agglomerated the red cells, and nourished any bacteria. Red cells settled to the bottom of the bag and were clamped off. The fluid was then passed through cellulose ester filters which collected any bacteria. The filters were placed on solid media for culture.	Growth was detected and microorganisms were identified faster with this method than with a broth method. Blood samples collected in an emergency can be held 24 hr in the plastic bags during which time the bacteria continue to grow. Also inhibitors were diluted by this method.		2-4 hr		6-7	Autoclave, blood collection app, plastic blood bags, plastic jaw sealer, clamps, cellulose ester filters, vacuum source, sterile forceps 5% aq glucose soln, Proteose Peptone No. 3, Tryptose, yeast ext, sodium chloride, disodium phosphate	Blood culturing	Kozub (1969)
381. Parathyroid gland	Fresh, newborn human parathyroid	A method was described for culturing parathyroid gland tissue from newborn humans in such a way that the tissue was gradually adapted from growth on an embryonic medium (consisting of plasma, cord serum, fetal brain press juice, and balanced saline) to growth on a medium extd from the future host. Such tissue was used for parathyroid transplantation especially in postoperative tetany.	After 14-21 days on the new medium the explants were considered 'adapted' and ready for transplantation. Results of transplants showed that only acceptors under 30 yr had a chance for recovery.		Total time not given; however, final adaptation to the host fluids took 10-21 days	Not given; it was implied that tissue could be continuously cultured for some time	Several	Surgical tools, culture dishes, embryonic watch glasses, incubator Various media	Parathyroid transplants	Gaillard (1954)
382. Sputum	Fresh human sputum	Sputum from a suspected tuberculosis patient was placed on sterile cotton swabs which were immersed in vials contg Cary-Blair medium and were then tightly capped. The vials were easily transported or kept in this fashion.	Samples examined by smear and culture were positive up to 23 days. Samples held from 51-154 days were negative on culture but positive for acid-fast bacilli on smear.	A patient diagnosed anywhere in the world and referred to a treatment center can bring an initial sputum sample with him using this technique. Also, availability of a pretreatment sample would allow a full study of drug sensitivities during treatment.	Few min	Up to 23 days	1-2	Glass vials, cotton swabs Cary-Blair medium	Culture and smear tests for diagnosis of bacterial diseases	Cohen (1967)

SPECIMEN DESCRIPTION	ORIGINAL FORM OF SAMPLE	BRIEF DESCRIPTION OF METHOD	LIMITATIONS OF METHOD	OTHER COMMENTS ABOUT METHOD	ESTIMATED PRESERVATION TIME	SURVIVAL TIME	NO. OF STEPS	EQUIPMENT AND REAGENTS USED	END USE OF SPECIMEN	REFERENCE
383. Tissue culture cells	Suspension of cells in fluid medium	Pure L strain of fixed tissue cells was successfully grown in fluid suspensions of circulating dilute horse serum, chick embryo ext, and 0.1% methylcellulose. The cells were discouraged from adhering to the container by rapidly rotating the tubes contg medium and cells. A history of techniques leading up to this technique was also given.	Viability of the fluid suspensions of these tissue cells was not given; however cells grown by previous methods mentioned were maintained from 6 mon to more than 10 yr depending on the type of cell and method used.	There was an initial conditioning period for the cells to modify the fluid medium before growth occurred.	Not given	From 6 mon to >10 yr depending on strain and method	<10	Multiple drum rotating or roller tube unit, tubes, refrigerator?, incubator? 0.1% Methocel, 4000 cps (Dow); dilute horse serum-chick embryo medium	Continuation of cell lines for research	Earle (1954)
384. Trypanosomes	6-day cultures of Trypanosoma lewisi grown on a diphasic modified medium	Trypanosoma lewisi, usually cultured in a complex diphasic agar medium, was instead successfully grown in the liq phase of a culture medium in which the blood agar components were placed inside a double layer of dialysis tubing, autoclaved, and immersed in Locke's soln. Agar appears to have an important function in the culture and reasons for this are discussed.	Viable organisms can be observed 3 mon postinoculation. They exhibit normal morphology and exponential growth between the first and fifth or sixth days. All free amino acids decrease in the dialysate medium during growth of the flagellates, except alanine, which increases. Ammonia conc also increases.		Not clear	3 mon	8-9	Pipettes, dialysis tubing, centrifuge, autoclave, 0.22 μ Millipore filter, Freas Model 805 incubator Bacto beef; Bacto peptone; NaCl soln; Bacto agar; double-distd, deionized H_2O, pH 7.2-7.4; defibrinated rabbit blood; Locke's soln	Culturing methods for Trypanosoma lewisi	Dusanic (1969)

ASHING

SPECIMEN DESCRIPTION	ORIGINAL FORM OF SAMPLE	BRIEF DESCRIPTION OF METHOD	LIMITATIONS OF METHOD	OTHER COMMENTS ABOUT METHOD	ESTIMATED PRESERVATION TIME	SURVIVAL TIME	NO. OF STEPS	EQUIPMENT AND REAGENTS USED	END USE OF SPECIMEN	REFERENCE
385. Biol samples	Mostly fresh samples	A combustion or ashing system was described for preparing large numbers of biol samples for scintillation counting. The system can be used for a variety of samples and includes a method for evaporating tissue homogenates in gelatin or plastic capsules. Capsules were dropped into a furnace and the combustion products were collected in scintillation solvent ready for counting. The system accepts samples of at least 500 mg and is limited by the solvent capacity for water. Samples may be dried or lyophilized right in the capsules before combustion, and they may be refrigerated after combustion until ready for counting.	The method compared favorably with the oxygen flask method but was much faster. It had a collection recovery of 96%, was calibrated by internal standards, and had a coefficient of variation of 2.9%.	Plastic capsules were preferred over gelatin ones, but they had to be specially made. Although oxygen was used in the system no hazardous incidents have occurred during its use.	Few min not counting sample drying or lyophilizing which can be done in batches	Not given	Varies with sample type	Combustion tube app (described), gelatin or plastic capsules, evaporation block, hot plate, vacuum system, dessicator, glass vials, refrigerator Silicone oil, formaldehyde, methanol-toluene or dioxane-toluene scintillation solvents, oxygen	Scintillation counting	Peterson (1969)
386. Mollusks	Fresh live mollusks	<u>Mytillus galloprovincialis</u> mollusks were allowed to take up radioisotopes of cerium, cobalt, manganese, proactinium, ruthenium, and zinc from sea water for 1-7 days. The molluscs were washed, and the soft parts were separated from the shells, placed in porcelain crucibles, weighed, and dried at 110°C. Then they were heated at temp from 250°-800°C for several hr to obtain constant activity in the sample. After heating, samples were cooled, and radioactivity was measured.	All of the trace elements studied were partly volatilized even at the low ashing temp; therefore wet ashing, rather than dry ashing, was recommended for detn of radioactive contaminants in biol material.	This particular mollusk was used for the expt because of the high biol conc factors of the investigated radionuclides.			6-7	Sea water aquarium, sea water, porcelain crucibles, oven, gamma ray spectrometer, analytical balance Radionuclides Mn-54, Co-56, -57, and -58, Zn-65, Ru-106, Ce-144, and Pa-233	Anal of trace minerals by a dry ashing technique	Strohal (1969)

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RADIATION

SPECIMEN DESCRIPTION	ORIGINAL FORM OF SAMPLE	BRIEF DESCRIPTION OF METHOD	LIMITATIONS OF METHOD	OTHER COMMENTS ABOUT METHOD	ESTIMATED PRESERVATION TIME	SURVIVAL TIME	NO. OF STEPS	EQUIPMENT AND REAGENTS USED	END USE OF SPECIMEN	REFERENCE
387. HeLa cells	Monolayer cultures of HeLa cells	HeLa cells in Eagle's medium with 20% pooled human serum and 10% DMSO were frozen in ampules to liq nitrogen temp. Some samples were gassed with CO_2 in white spot nitrogen to make them hypoxic, and others were irradiated at -196°C with x-rays at the rate of 100 rads/min acute radiation or with Co^{60} gamma rays at the rate of 100 rads/day continuous radiation.	Acute x-ray radiation caused a decrease in the slope of the HeLa cell survival curve. Addition of DMSO, lowering the radiation temp to -196°C, and rendering the HeLa cells hypoxic indicated successive dose modifying factors. The D^{o} rose from 149 rads at room temp to 963 rads when cells were subjected to all three of these dose modifying factors.	There was an oxygen effect with respect to the quality of cells produced after irradiation, since oxygen apparently combined with free radicals formed during irradiation.	Varies with method	Not given	5-8	Pipettes, ampules, culture bottles, degassing app, Linde biol freezer (Type BF5), Union Carbide liq nitrogen refrigerator, heat sealer, Dewar flask, Resomax x-ray machine, Co^{60} source Eagle's medium contg 20% pooled serum and 10% DMSO, 5% CO_2 in air or nitrogen	Study of fundamental action of radiation on a biol system	Nias (1969)
388. Spores	Freshly cultured bacterial spores	Clostridium botulinum strain 33A spores which were resistant to gamma radiation, UV radiation and heat, were cultured in trypticase-peptone broth. Equal vol of spores and borate buffer were combined to give a pH range of 6-12. Samples were pipetted into pyrex tubes, sealed, and frozen to 0°C, -20°C, or -196°C. Then they were irradiated with 0.6-0.9 Mrad of Co-60. After irradiation, samples were thawed and analysed for surviving spores.	Radiation resulted in a wavelike pattern of survival influenced by pH, temp, and radiation dose. Three patterns were recognized: A, at -190°C showed peaks of high survival at pH 7 and 9.5 and troughs of low survival at pH 8 and 10-11; B, at -50°C showed high survival at pH 8.5-9 and low survival at pH 7 and 10; C, at 0°C showed high survival at pH 7.5 and 10 and low survival at pH 6, 9, and 11. An explanation was presented.		Not clear	Samples were thawed immediately	5-7	Oval culture tubes, pipettes, autoclave, pyrex tubes, freezer, radiation source 5% trypticase-0.5% peptone broth, Wynne's broth, agar, sterile borate buffer, dry ice, borate buffer, phosphate buffer	Sterilization using radiation	Upadhyay (1969)

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DIALYSIS

SPECIMEN DESCRIPTION	ORIGINAL FORM OF SAMPLE	BRIEF DESCRIPTION OF METHOD	LIMITATIONS OF METHOD	OTHER COMMENTS ABOUT METHOD	ESTIMATED PRESERVATION TIME	SURVIVAL TIME	NO. OF STEPS	EQUIPMENT AND REAGENTS USED	END USE OF SPECIMEN	REFERENCE
389. Milk dialysate	Fresh whole milk	Fresh whole milk was centrifuged in sterile centrifuge bottles at $5^{\circ}C$. Skim milk was removed with a sterile pipette, recentrifuged and the skim-milk supernatant passed through both 1 and 45 μ sterilized Seitz filters. A sterile dialysis bag contg 250 ml of sterile distd water was immersed completely in 625 ml of the milk filtrate in an Erlenmeyer flask and was dialyzed for 2 days at 0°-$4^{\circ}C$. The skim milk was replaced with a fresh vol of raw skim milk, and dialysis was continued for one more day. The contents of the dialysis bag were collected aseptically in a sterile Erlenmeyer flask. Total vol was 120 ml.	No bacterial growth occurred on Standard Plate Count Agar after incubation of the milk dialysate at $35^{\circ}C$ for 72 hr. The dialysate could be stored several wk at $4^{\circ}C$ and was free of protein.	Milk dialysate is used as a native protein-free milk buffer in protein research.	3-4 days	Several wk	9-10	Dialysis bag, autoclave, sterile centrifuge bottles, sterile pipettes, 1 and 0.45 μ Seitz filters, Erlenmeyer flasks	For use as a native protein-free milk buffer in protein research	Koka (1967)

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INCUBATION

SPECIMEN DESCRIPTION	ORIGINAL FORM OF SAMPLE	BRIEF DESCRIPTION OF METHOD	LIMITATIONS OF METHOD	OTHER COMMENTS ABOUT METHOD	ESTIMATED PRESERVATION TIME	SURVIVAL TIME	NO. OF STEPS	EQUIPMENT AND REAGENTS USED	END USE OF SPECIMEN	REFERENCE
390. Blood plasma	USP human blood plasma	USP human plasma was found to have unusually high levels of free fatty acids ranging from 2,889-3,272 μeq/l. During prepn plasma was kept at 32°C for several mon to inactivate hepatitis virus. An investigation was made to see if the long incubation time was the cause of the high fatty acid content.	The long incubation period for USP human plasma apparently caused the increase in free fatty acids. Reasons for this were discussed. 0.6 M sodium chloride reduced the release of free fatty acids during incubation.	The authors suspected that lipoprotein lipase liberated the free fatty acid but this was not proven.	Several wk including incubation	Not given		0.6 M NaCl	Prepn of USP blood plasma	Barboriak (1968)

IV. INDEX TO TABLES

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